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BLOOD SUGAR, INORGANIC PHOSPHORUS AND PHOSPHATASE ACTIVITY FOLLOWING THE INTRAVENOUS INJECTION OF CALCIUM SALTS

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Many studies of the effect of calcium upon the level of blood sugar and upon glucose tolerance have yielded contradictory findings. These discordant results have been obtained in studies of the influence of calcium on epinephrine hyperglycemia and glycosuria (1, 2, 3), diabetic glycemia and glycosuria (4, 5), glucose tolerance (6) and in investigations of the effect of parathyroidectomy and parathyroid hormone injection upon blood sugar and sugar tolerance (7, 8, 9, 10, 11). Several theoretical objections can be raised to the adequacy of the experimental methods employed in many of these studies on the basis of 1, the animal species employed; 2, the nature of the calcium salt administered; 3, the notable variability observed in repeated glucose tolerance tests; 4, the well-recognized variability of response of different species and different individuals to the injection of parathyroid extract, and 5, the frequent failure to make observations at the time of maximum blood calcium concentrations.

METHODS. The present studies were made upon 8 trained, adult dogs, ranging from 10 to 21 kgm. in weight, 24 hours after the last previous feeding. The quantity of calcium administered ranged from 3.7 to 9.9 mgm. of Ca ion per kilogram of body weight, in the form of calcium gluconate or calcium levulinate,[1] injected into a leg vein. Blood was withdrawn from the jugular vein, without stasis, immediately before and 5, 15, 30 and 45 minutes after injecting the calcium salt. With the exception of slight tremors and retching in one animal, no untoward reaction was noted other than evidence of slight, transitory nausea, the animals lying quietly during the experimental period.

[1] The Sandoz Chemical Co. kindly supplied the calcium gluconate and the Crookes Co. the calcium levulinate.

TABLE 1

Serum calcium, phosphorus (inorganic), protein, phosphatase activity and sugar before and after the intravenous injection of calcium gluconate and calcium levulinate

Values for calcium, phosphorus and sugar expressed in milligrams per 100 cc., protein in grams per 100 cc. and phosphatase activity in Bodansky units per 100 cc. Figures in parenthesis represent values corrected for plasma concentration or dilution on the basis of changes in the concentration of serum proteins.

DOG	DOSAGE		MINUTES AFTER INJECTION				
			0	5	15	30	45
	mgm. Ca per kgm.						
1	9.9	Calcium	9.8	21.2	15.8	14.9	14.4
		Sugar	68	68 (61)	67 (58)	67	71
		Phosphorus	3.2	2.8 (2.5)	2.9 (2.5)	2.6	2.8
		Phosphatase	2.2	2.2	2.5	2.3	2.8
		Protein	6.2	6.8	7.0		6.3
2	9.9	Calcium	10.3	20.6	16.3	15.6	14.1
		Sugar	76	83	81	81	83
		Phosphorus	3.9	3.4	3.1	2.8	3.1
		Phosphatase	2.0	2.3	2.2	2.7	2.6
3	9.0	Calcium	10.2	21.1			14.3
		Sugar	89	80 (71)	68 (61)	72	78
		Phosphorus	3.7	3.1 (2.7)	3.3 (3.0)	2.8	2.8
		Phosphatase	2.4	2.8	3.5	2.7	2.8
		Protein	6.1	6.9	6.7	6.2	6.3
4	9.0	Calcium	9.7	20.6		16.4	13.1
		Sugar	98	90 (81)	81 (67)	83	76
		Phosphorus	2.6	2.1 (1.9)	2.0 (1.7)	2.2	2.5
		Phosphatase	3.1	3.3	3.7	3.7	3.5
		Protein	6.2	6.8	7.3		6.3
5	6.6	Calcium		20.2			14.8
		Sugar	72	63	60 (53)	65 (63)	71 (74)
		Phosphorus	2.7	2.8	3.1 (2.8)	2.7 (2.6)	2.7 (2.9)
		Phosphatase	3.7	2.49	3.25	3.21	2 91
		Protein	5.8	5.9	6.5	6.1	5.6
6	6.6	Sugar	71	63	66	63	67
		Phosphorus	4.1	4.1	3.5	3.8	4.2
		Phosphatase	5.75	4.81	5.88	5.54	5.75
7	6.6	Calcium	11.1	20.6			15.2
		Sugar	101	96	93	81	76
		Phosphorus	1.9	1.8	1.8	1.6	1.7
		Phosphatase	1.16	1.37	1.45	0.98	1.67
8	3.7	Calcium	10.4			17.3	15.8
		Sugar	96	83	65	85	76
		Phosphorus	2.7			2.5	2.5
		Phosphatase	1.72			1.81	1.84

Serum calcium was determined by the method of Clark and Collip (12), inorganic phosphorus by the method of Youngburg and Youngburg (13), serum protein by the method of Howe (14), serum phosphatase activity by the method of Bodansky (15) and sugar by the method of Benedict (16).

RESULTS AND DISCUSSION. The findings are presented in table 1. Following the injection of calcium some difficulty was at times experienced in removing sufficient blood for analysis, apparently because of a rather marked increase in its viscosity and coagulability. Red cell sedimentation was also observed to be accelerated in samples withdrawn after the injection. In the 4 cases in which the serum protein concentration was determined a maximum increase of 11.2 to 17.7 per cent above the control level was found in the 5 and 15 minute samples. Assuming that the observed variations in serum protein concentration are dependent upon alterations in plasma concentration, correction of the observed sugar and phosphorus values for such alterations should afford a more exact indication of the true behavior of these constituents than is obtained from the observed values themselves. These corrected values are indicated in the table in parenthesis.

With the exception of dog 2, in which the blood sugar concentration rose slightly but in which no observations were made regarding possible changes in plasma concentration, the blood sugar level decreased in each instance. When correction was applied in 4 cases for alteration in plasma concentration, on the basis of changes in the serum protein concentration, the maximum drop in each instance ranged from 10 to 31 mgm. per 100 cc. and occurred 15 minutes following the injection of calcium.

The serum inorganic phosphorus was consistently decreased following the injection, and the phosphatase activity showed variable and relatively insignificant deviations from the control levels. The decrease in phosphorus generally persisted after the concentration of sugar had begun to increase, and was well marked in dog 2, in which no diminution in blood sugar was detected.

The significance of these observations is conjecturable. It would appear, however, that hypercalcemia, per se, is not necessarily accompanied by an increase in blood sugar, as has been intimated by previous investigators, and that contradictory observations may perhaps be dependent upon other factors. Salvesen, Hastings and MacIntosh (17) and Collip (18) have reported an increase in serum inorganic phosphate following the injection of other salts of calcium, and a slight rise was noted by Greville (19) 4 to 6 minutes after the injection of calcium levulinate in rabbits. Although the reason for this discrepancy is not readily apparent, it should be pointed out that the most pronounced fall in phosphorus in the present experiment usually occurred in the sample withdrawn 30 minutes following the injection of calcium. On the basis of the data presented here, it is

obviously not justifiable to assume any direct relationship between the observed changes in blood sugar and inorganic phosphorus. It may be observed, however, that the fall in the latter is in accord with the hypothesis of increased glucose utilization as the basis for the diminished blood sugar content.

SUMMARY

The intravenous injection of calcium gluconate and calcium levulinate in doses of 3.7 to 9.9 mgm. Ca ion per kilogram of body weight was followed by 1, an 11.2–17.7 per cent increase in the concentration of serum protein; 2, a decrease of 10 to 31 mgm. per 100 cc. in blood sugar within 5 to 15 minutes; 3, a more gradual diminution in serum inorganic phosphorus, and 4, variable and insignificant alterations in serum phosphatase activity.

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AN IMPROVED GASTRIC TEST MEAL AND A STUDY OF THE SECRETORY CURVE IN WHOLE STOMACH POUCHES AND IN THE NORMAL INTACT STOMACH

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In previous publications (1, 2, 3, 4, 5, 6, 7, 8) the results of studies planned primarily to investigate the amount and the composition of the various secretions which may enter the stomach during the course of normal digestion have been reported. In these previous studies an acid test meal consisting of approximately tenth normal hydrochloric acid containing 11 mgm. of the sodium salt of phenol red per liter was used with and without histamine stimulation. By means of methods and calculations which were described in detail it was possible to determine with a fairly high degree of accuracy, the amount and strength of the acid secretion and the amount and composition of the non-acid secretions of both intragastric and duodenal origin. In these studies no definite conclusions were drawn regarding the exact rôle of the various secretions in the regulation of gastric acidity. In the present communication we wish first, to describe a test meal which appears to be very satisfactory for use in experimental studies, and second, to show by means of studies on whole stomach pouches and the intact normal stomach, using this test meal, how the various secretions entering the stomach interact to regulate gastric acidity.

The test meal. We attempted to develop a test meal having the following characteristics: 1. High secretagogue effect so that the use of histamine or other extraneous stimulants would be unnecessary. 2. One which would permit the use of phenol red to determine the factor of dilution, as described by Gorham (9) and used by us in our previous studies. 3. One which could be accurately standardized. 4. One which would have no acid combining or acid neutralizing properties.

Several substances (milk, whey, Armour's meat extract) were tried but found unsatisfactory A two per cent solution of Liebig's meat extract (Lemco brand) was more satisfactory and was adopted for use. A study of unmodified, two per cent aqueous solutions showed that they are not satisfactory where a high degree of accuracy is desired, for the following reasons: 1. Non-volatile acids are present which may interfere with the determina-

tion of neutral chloride in the gastric samples. 2. When acidified with tenth normal hydrochloric acid a heavy flocculent precipitate forms which is redissolved in excess acid. 3. They possess a variable ability to neutralize and combine with hydrochloric acid. A two per cent solution was therefore prepared in which these undesirable features were eliminated.

Preparation of 2 liters of standard 2 percent Liebig's extract test meal containing 15 mgm. of phenol red per liter. 1. Forty grams of Liebig's extract dissolved in 1 liter of distilled water. The solution may be warmed to facilitate solution but care should be taken never to approach the boiling point since this may destroy the secretagogue (10).

2. Add 30 mgm. of the sodium salt of phenol red.

3. Add 20 cc. of 20 per cent sodium carbonate solution. The solution should then be definitely alkaline as shown by the red color of the phenol red.

4. Add slowly with constant stirring, one liter of tenth normal hydrochloric acid. After the addition the solution should be definitely acid to litmus paper.

5. Let stand until a heavy flocculent precipitate settles.

6. Filter. The first portions that pass through will be turbid and should be returned to the filter until the filtrate is crystal clear.

This meal usually contains an average of approximately 220 mgm. of total chloride, 180 mgm. of neutral chloride and 40 mgm. of acid chloride per 100 cc. When titrated with bromcresol purple, the acid concentration usually ranges between 13 and 18 cc. of N/10 acid per 100 cc. If a constant procedure is followed in preparation, different batches of the meal will have a fairly constant composition. We have routinely made a complete analysis of the meal each time that it was used. Sufficient quantity of meal can be made for from 7 to 10 days' use and kept in a refrigerator. The taste of this meal is quite agreeable and it can readily be taken by mouth.

METHODS. The general management of the animals prior to and during the test was the same as that described in previous publications. A light feeding of milk and Karo syrup was given approximately 24 hours before the test. The fractional method of analysis was used, samples being removed every half hour until the stomach emptied. Before beginning a test the stomach was lavaged with 300 cc. of the test meal in order to remove accumulated secretions or water.

Since we wished to closely simulate conditions existing during digestion of a meal it was desired to have some solid particles in the stomach along with the Liebig's extract test meal. Several uncooked vegetables (rice, corn, beans, wheat) and also bread were tried but it was found that all adsorbed or absorbed phenol red from the test meal in the stomach and could not be used. Glass beads were finally used to supply the solid portion of the meal. The beads were placed in the mouth and a small amount

of meal poured in. From three to four tablespoonsful were used and they were swallowed without chewing. Beads were used in all experiments on 4 dogs and omitted in all experiments on 7 dogs, but no clear cut differences were noted in the two groups, so that it may be concluded that the presence of solid materials in the stomach causes little change in the secretory curve.

It was found necessary to ash the gastric samples before determining the content of chloride, since the test meal contains sufficient organic material to interfere with the quantitative precipitation of chloride by silver and thus render the results grossly inaccurate. Total and neutral chloride were determined as described in a previous publication (1). In the determination of total chloride 0.3 cc. of a saturated solution of sodium nitrate was added to facilitate ashing. This was not used in the determination of neutral chloride.

In determining the per cent of phenol red in the gastric samples, 5 cc. quantities of the original test meal and of each of the gastric samples were used. Two cubic centimeters of 20 per cent sodium tungstate and 2 cc. of 1.33 normal sulfuric acid were added to each, and the contents mixed by inverting, without shaking. The tubes were then corked and allowed to stand for from 1 to 1½ hour. During this period a heavy flocculent precipitate settled out, usually leaving a clear supernatant liquid. The precipitate does not remove phenol red by adsorption. The samples were next centrifuged at high speed for about 10 minutes and the supernatant fluid decanted into graduated 15 cc. centrifuge tubes. The volumes in all tubes were reduced to 8 cc. and alkalinized by adding 1 cc. of approximately 20 per cent sodium hydroxide. The contents were then mixed and centrifuged at high speed for about 10 minutes. The gastric samples were then compared in a colorimeter with the sample of the original test meal. It was found best to set the standard at 5 mm. and make the comparison on this basis.

Immediately after alkalinizing the samples usually assume a purplish red color different from the true color of alkaline phenol red. This is due to certain substances in the meat extract (uric acid, tyrosine, tryptophane?) which react with the excess tungstate in an alkaline media to produce a blue color. This blue color fades rapidly, however, and is also adsorbed on the slight precipitate thrown down.

If the dilution has been very marked or large amounts of duodenal contents are present in the sample it frequently happens that the treatment with sodium tungstate and sulfuric acid does not yield a perfectly clear supernatant fluid but a milky one which cannot be cleared by centrifuging. However, the samples will clear after addition of the alkali and are satisfactory for comparison.

Two additional manipulations are often necessary to insure satisfactory determination of the per cent of phenol red. These are as follows:

1. When an experiment is continued until complete emptying of the

stomach occurs, it is usually found that the last samples are too dilute to determine the per cent of phenol red accurately and also too dilute to clear properly. We routinely made an accurate dilution of such samples with equal parts of the original test meal and then made all determinations, except that for bile, on the diluted samples. Since the composition of the test meal was accurately known, a simple correction gave the true composition of the gastric sample. With a few exceptions we diluted all samples from 1½ hour on. If one tries to employ a higher original concentration of phenol red in the test meal in order to have sufficient color for the final samples, then it is found that the color is too deep for accurate determination in the earlier samples. The method of accurate dilution and proper correction has been found perfectly satisfactory.

2. When large amounts of bile, especially gall bladder bile are present in the sample, the color after alkalinization is often faintly tinged with yellow. This color can be almost perfectly matched by adding a few small cyrstals of picric acid to a small amount of the standard. By mixing the picric standard with varying amounts of the untreated standard a yellowish tinge can be produced which almost exactly matches that of the gastric sample.

Each gastric sample was tested for bile using a Pettenkofer ring test. The amount of bile was read as from zero to 4 plus depending on the width and depth of color of the ring. The meal does not give false positive reactions.

Calculations. The general principle of the calculations is as follows: The decrease in the per cent of phenol red in the sample removed from the stomach is due to dilution of the test meal by the various fluid secretions entering the stomach. If the fluid which diluted the test meal had been a neutral fluid containing no chloride, then the total, neutral and acid chloride in the gastric sample would be equal to the corresponding amounts in the original test meal multiplied by the per cent of phenol red in the gastric sample. However, if neutral and acid chloride were present in the fluid secretions which diluted the test meal the amounts added will be the difference between the amount in the original test meal (corrected for dilution) and the amount in the gastric sample. The details are as follows:

a. *Extra total chloride.* The total chloride in the gastric sample minus (total chloride of the test meal multiplied by the per cent of phenol red in the gastric sample) equals the extra total chloride in the gastric sample. This represents the total chloride added by all the secretions which entered the stomach and were mixed with the test meal and may include secreted hydrochloric acid and the neutral chloride present in mucus, pyloric secretions and regurgitated duodenal secretions. If the extra total chloride is divided by the number of cubic centimeters of secretion in the gastric sample (decrease in the per cent of phenol red) and multiplied by

100 the result will give the total chloride concentration per 100 cc. of the mixed secretions entering the stomach.

b. *Extra neutral chloride.* The neutral chloride in the gastric sample minus (neutral chloride of the test meal multiplied by the per cent of phenol red in the gastric sample) equals the extra neutral chloride in the gastric sample. This may include neutral chloride present in gastric mucus, pyloric secretions and regurgitated duodenal secretions as well as neutral chloride resulting from the neutralization of hydrochloric acid. The neutral chloride concentration per 100 cc. of mixed secretions entering the stomach is calculated as explained above.

c. *Extra acid chloride.* The extra total chloride minus the extra neutral chloride equals the extra acid chloride. This represents the acid which was secreted by the stomach and not neutralized. The acid chloride concentration per 100 cc. of the mixed secretions entering the stomach is obtained as explained above.

A further calculation which has been found of great value is the division of the total fluid entering the stomach into the *fluid of the acid which was secreted and not neutralized* and the *non acid* or *extra fluid.* This calculation is based on the fact that the strength of the acid secreted by the fundic glands remains practically constant regardless of the amount secreted (3, 12, 13, 14, 15, 16, 17).

1. *Total fluid.* The decrease in the per cent of phenol red in the gastric sample shows the number of cubic centimeters of fluid entering the stomach and mixed with each 100 cc. of test meal. This total fluid is composed of acid and non-acid fluid.

2. *The acid fluid.* The work of several recent investigators (1, 4, 18, 19) has shown that the acid as secreted by the fundic cells contains approximately 600 mgm. of acid chloride per 100 cc. and no neutral chloride. If the milligrams of extra acid chloride in the gastric sample are divided by 6.0 the quotient will represent the cubic centimeters of acid fluid per 100 cc. of gastric sample.

3. *Non-acid or extra fluid.* This is the difference between the total fluid and the acid fluid and may include the non-acid secretions of the pyloric and fundic regions, the fluid of regurgitated duodenal secretions and the fluid of hydrochloric acid which was secreted and subsequently neutralized.

The non-acid secretions of the stomach and the regurgitated duodenal secretions all contain neutral chloride (300 to 378 mgm. per 100 cc.) (2, 3, 4, 5, 6) and may also neutralize small amounts of hydrochloric acid with the production of further neutral chloride. Thus it is evident that the extra neutral chloride in the gastric sample is entirely related to the *non-acid* or *extra fluid.* If the milligrams of extra neutral chloride are divided by the cubic centimeters of extra fluid the result will give the neutral chloride-extra fluid ratio which is often of importance in analyzing the results.

RESULTS. A. *Whole stomach pouches*: Four whole stomach pouches of different types were studied. In pouch I the duodenum was severed from the pylorus and both were closed. The esophagus with a small part of the cardiac end of the stomach was anastomosed, end to side, to the duodenum. The whole stomach pouch was opened to the outside by a gastrostomy. In this type of pouch most of the vagus fibers were cut. This animal was fed as usual and remained in excellent condition for several months. Pouch II was made by a two stage operation. At the first operation a non-leaking fistula of the Mann-Bollman type was placed on the lower duodenum. Several weeks were allowed to elapse before the second operation at which the duodenum was separtated from the pylorus and both were closed. At the same time the stomach was opened to the outside by a gastrostomy. Feeding was carried out through the duodenal fistula and daily subcutaneous injections of saline (1000 cc.) were given. No experiments were performed before the pyloric portion of the stomach had healed. Pouches III and IV were also made by a two stage technique. At the first operation the pylorus was separated from the duodenum and both were closed. At the same time an anterior gastrojejunostomy with a small stoma was made. Several weeks were allowed for recovery. At the second operation the jejunostomy was removed, the opening in the jejunum closed and the opening in the stomach brought to the surface as a gastrostomy opening. Experiments were performed on the 2nd, 3rd and 4th days after the second operation. No food was given but 1000 cc. of saline were given subcutaneously daily after completion of the experiment. Pouches II, III, and IV were isolated from the intestine but attached to the esophagus and the nerve supply was intact. No essential differences were noted in the results obtained on these different types of pouches. All animals were in satisfactory condition when the experiments were performed.

The results of fractional analyses are shown in table 1 and an average secretory curve for all experiments is shown in the lower part of figure 1. The following features are of special importance:

1. *The extra or non-acid fluid (col. 14).* The extra or non-acid fluid was in general very small in amount. The largest amount was usually present in the first sample after which it decreased and finally disappeared. In previous studies on whole stomach pouches in which a tenth normal hydrochloric acid test meal and histamine stimulation was used (4) considerably larger amounts of extra fluid were found, the average secretion rate being about 6 to 8 cc. per half-hour, this rate remaining fairly constant for as long as two hours. Since tenth normal hydrochloric acid is a definite stimulant for the pyloric secretions (20) it was expected that less extra fluid would be found when the Liebig's extract test meal was used but its complete absence in so large a number of experiments was quite surpris-

TABLE 1

Fractional experiments on four whole stomach pouches of different types

POUCH	COMPOSITION OF TEST MEAL		GASTRIC SAMPLE: Total chloride	GASTRIC SAMPLE: Neutral chloride	GASTRIC SAMPLE: P.S.P.	EXTRA TOTAL CHLORIDE	EXTRA NEUTRAL CHLORIDE	EXTRA ACID CHLORIDE	CHLORIDE CONCENTRATION OF GASTRIC SECRETION: Total	CHLORIDE CONCENTRATION OF GASTRIC SECRETION: Neutral	CHLORIDE CONCENTRATION OF GASTRIC SECRETION: Acid	TOTAL FLUID	ACID FLUID	EXTRA FLUID	TIME	REMARKS
		mg. per 100 cc.	*mg. per 100 cc.*	*mg. per 100 cc.*	*per cent*										*hrs*	
I	Total	217	240	177	94	36	11	25	600	183	417	6	4	2	½	Partially (vagus) denervated. Esophagus anastomosed to duodenum
	Neutral	177	272	179	90	77	20	57	770	200	570	10	10	2	1	
	Acid	40	320	186	85	136	36	100	907	240	667	15	17	0	1½	
			374	186	74	213	55	158	820	212	608	26	26	0	2	
I	Total	218	244	168	93	41	15	26	585	214	371	7	4	3	½	
	Neutral	164	279	172	89	85	26	59	773	236	537	11	10	1	1	
	Acid	54	324	161	82	145	26	119	806	145	661	18	20	0	1½	
			367	151	75	204	28	176	816	112	704	25	29	0	2	
I	Total	218	233	168	95	26	7	19	520	140	380	5	3	2	½	
	Neutral	170	254	173	93	51	15	36	728	215	513	7	6	1	1	
	Acid	48	285	168	90	89	15	74	890	150	740	10	12	0	1½	
			323	152	88	131	2	129	1092	17	1075*	12	22	0	2	
I	Total	214	237	174	95	33	12	21	660	240	420	5	4	1	½	"Block" experiment with 200 cc. each half-hour
	Neutral	170	246	173	92	49	16	33	613	200	413	8	6	2	½	
	Acid	44	245	168	93	46	10	36	656	143	513	7	6	1	½	
			238	179	93	39	21	18	558	300	258	7	3	4	½	
II	Total	220	260	183	91	60	14	46	666	155	511	9	8	1	½	Non-denervated pouch with non-leaking duodenal fistula
	Neutral	186	291	177	86	102	17	85	729	122	607	14	14	0	1	
	Acid	34	326	188	79	152	41	111	724	195	529	21	19	2	1½	
			357	202	74	194	64	130	746	246	500	26	22	4	2	
II	Total	222	270	182	89	72	12	60	655	109	546	11	10	1	½	
	Neutral	191	327	172	76	158	27	131	659	113	546	24	22	2	1	
	Acid	31	366	173	70	211	39	172	703	130	573	30	29	1	1½	
			404	163	65	260	39	221	744	111	633	35	37	0	2	
III	Total	215	228	177	93	28	16	12	400	228	172	7	2	5	½	Non-denervated, without duodenal fistula
	Neutral	173	263	166	91	67	9	58	744	100	644	9	10	0	1	
	Acid	42	296	163	85	113	16	97	753	107	646	15	16	0	1½	
			350	164	78	182	29	153	826	132	694	22	25	0	2	
III	Total	214	251	177	91	56	21	35	623	233	390	9	6	3	½	
	Neutral	171	270	174	89	79	22	57	719	200	519	11	10	1	1	
	Acid	43	291	164	88	103	14	89	860	117	743	12	15	0	1½	
			367	159	83	189	17	172	1112	100	1012*	17	29	0	2	
IV	Total	223	263	182	89	65	19	46	591	173	418	11	8	3	½	Non-denervated, without duodenal fistula
	Neutral	183	309	165	82	126	15	111	700	83	617	18	18	0	1	
	Acid	40	344	187	77	172	45	127	747	196	551	23	21	2	1½	
			394	155	72	233	23	210	831	82	749	28	35	0	2	
IV	Total	221	268	173	91	67	6	61	745	67	678	9	10	0	½	
	Neutral	183	308	170	82	127	20	107	705	111	594	18	18	0		
	Acid	38	343	161	78	171	18	153	777	82	695	22	26	0	1½	
			372	175	76	204	36	168	850	150	700	24	28	0	2	

Average of 38 samples 553
Average of 18 samples with zero extra fluid 664

* Not included in average.

ing. It appears that this is due not to a failure of secretion of non-acid fluid by the pouch but to a reabsorption of the fluid (water) as the experiment progresses. A "Block" type of experiment in which 200 cc. of meal were placed in the pouch and allowed to remain for one half hour and then completely removed, this being repeated every half hour for two hours, was performed on several occasions. In the example given in pouch I it is seen that the extra or non-acid fluid does not tend to disappear as the experiment progresses but actually shows a slight increase. This shows that the tendency for water absorption is dependent on the time factor, hence it is more pronounced in fractional than in "Block" types of experiments.

2. *The acid chloride.* As shown in column 8 there was a copious secretion of acid by the pouches. The acid chloride concentration of the secretion (col. 11) is seen to be low in the first sample but to rise progressively as the experiment progresses reaching its highest value in the last sample. This behavior is therefore the reverse of that of the non-acid or extra fluid. The average value for all 38 samples shown in table 1 is 553 mgm. of acid chloride per 100 cc. of secretion while in 18 samples in which no extra fluid was present the value is 664 mgm. per 100 cc. As pointed out above it has been well established by the work of several recent investigators that the acid chloride concentration of the pure acid secretion is approximately 600 mgm. of acid chloride per 100 cc. The average value of 664 mgm. per 100 cc. in the samples in which no extra fluid was present is further evidence of the absorption of small amounts of water from the stomach. This is very evident in the last sample in experiment 3, pouch I and the last sample in experiment 2, pouch III. Again it is found that the amount of water absorbed is related to the length of time that the meal remains in the stomach, since in the "Block" type of experiment in which the samples remained in the stomach for only one-half hour, there was little evidence of absorption.

Wilhelmj, Henrich and Hill (4) found that when a test meal of tenth normal hydrochloric acid was used with or without histamine stimulation, there was no evidence of absorption of water from the pouch, even in fractional types of experiments. It is therefore possible that the initial degree of acidity of the gastric contents is a factor in determining whether or not water absorption will occur.

The present experiments do not lend support to the theory (21) that hydrochloric acid, as such, is absorbed from the stomach. It appears that when once acid has been secreted by the isolated stomach, it remains there, no detectable amount being absorbed and only small amounts being neutralized.

3. *The total chloride concentration of the secretion (col. 9).* The total chloride concentration of the gastric secretions is determined by the relative amounts of acid and non-acid fluid (3, 4, 6). Since the pure acid

fluid has a chloride concentration of 600 mgm. per 100 cc. and the non-acid fluid a neutral chloride concentration of from 300 to 378 mgm. per 100 cc. it is clear that the total chloride concentration should not exceed 600 mgm. per 100 cc. As seen in column 9 figures above this value are very common, thus offering further evidence of water absorption from the pouches.

4. *The neutral chloride.* The extra neutral chloride is shown in column 7 and the neutral chloride concentration of the gastric secretion in column 10. The extra neutral chloride is small in amount and may either remain nearly constant, rise slightly or decrease, a slight rise being the most common behavior. The neutral chloride concentration of the secretion also behaves irregularly but usually decreases as the experiment progresses.

These experiments show quite clearly that when a Liebig extract test meal is used in the isolated whole stomach the amount of non-acid fluid and the neutral chloride are both very small in amount and that the acid chloride concentration of the secretion rises progressively throughout the experiment. They also demonstrate that the absorption of water may be a factor in concentrating the acid after it has been secreted.

B. *The intact normal stomach:* The present analysis is based on 67 experiments performed on 11 normal animals.

1. *The emptying time.* The emptying time of the stomach determined the duration of the experiment, and varied from $1\frac{1}{2}$ to $2\frac{1}{2}$ hours with a few 1 and 3 hour experiments. When the same amount of meal was given, the same animal was fairly constant in the emptying time so that we can speak of a slow or fast emptying time as being characteristic of a given animal. The presence of solid material in the stomach (beads) seemed to produce some prolongation of the emptying time although some animals in which beads were not used emptied as slowly as others in which beads were used.

2. *The secretory curves.* An analysis of 67 experiments shows that the secretory curves can be divided into five types, clear cut examples of which are shown in the upper part of figure 1. The frequency of these different types was as follows:

Type I	42 or 63 per cent
Type II	12 or 19 per cent
Type III	7 or 10 per cent
Type IV	3 or 4 per cent
Type V	3 or 4 per cent

Types I and II, which are very similar, comprise 82 per cent of the total number and can therefore be spoken of as the *common normal types* of curves.

In all types of curves the *total fluid* entering the stomach increased progressively until emptying occurred. The relative amounts of acid and non-

acid fluid, however, showed certain changes during the experiment and the nature of these relative changes determined the type of the curve. It is important to remember that the acid chloride concentration of the total

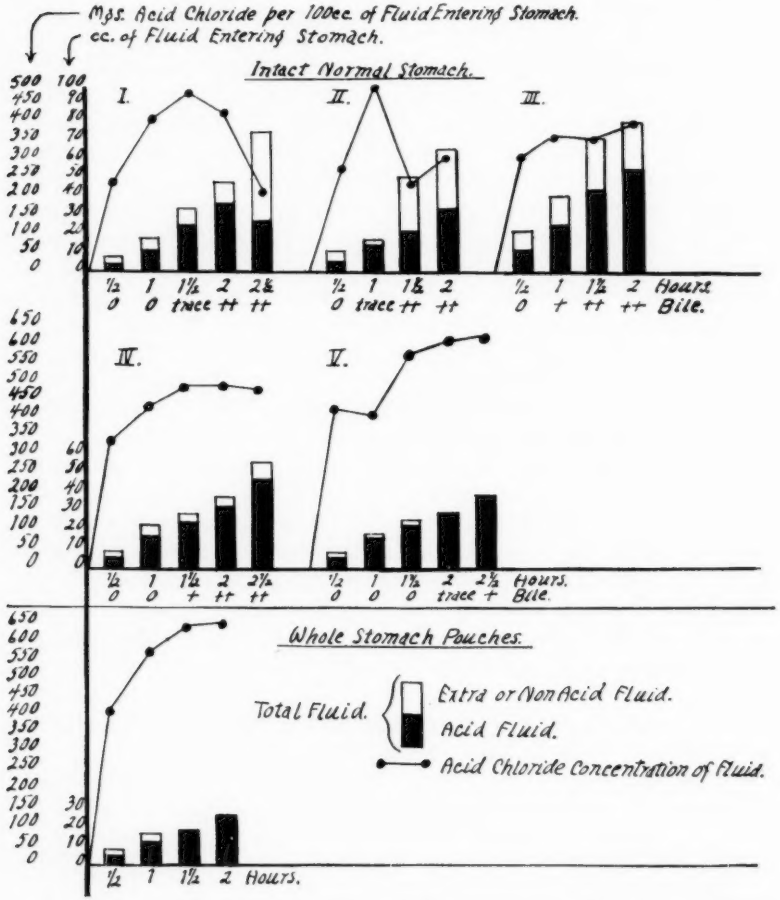

Fig. 1. Upper half shows the five types of secretory curves obtained on normal dogs. Types I, IV and V are from the same dog.

Lower half, an average secretory curve from 10 experiments on four whole stomach pouches.

fluid entering the stomach depends upon the *relative* (not the absolute) amount of acid and non-acid fluid. For example, both the acid and non-acid fluids may show a sharp increase in a given sample, but the acid chloride

concentration of the total fluid may either rise or fall depending upon whether the increase in acid fluid was relatively greater or less than the increase in the extra fluid.

In curves of type I the changes in the relative amounts of acid and non-acid fluid can, in general, be divided into three phases. During the *first phase* which comprises the first half hour, the extra fluid is relatively (not absolutely) high in relation to the acid fluid, due largely to the slow start of the acid secretion. Bile is practically never present and the non-acid fluid is probably mostly of intra-gastric origin. The *second phase* which comprises the next one or two half hour samples, depending upon the emptying time, is characterized by a rapid relative increase in the acid fluid and a corresponding relative decrease in the extra fluid. Bile is usually present in small amounts so that the non-acid fluid is of both intra and extra gastric origin. The *third phase* which comprises the last one or two half-hour samples before emptying occurs, is characterized by an abrupt relative increase in extra fluid with a corresponding decrease in acid fluid. Bile was always present during this phase.

The acid chloride concentration of the total fluid entering the stomach shows corresponding changes during the three phases; rising to a low value during the first phase, rising rapidly to a variable height during the second phase and decreasing during the third phase.

Curves of type II are very similar to those of type I except that in the last sample there is a small relative increase in the acid fluid with a corresponding slight secondary rise in the acid chloride concentration of the total fluid.

In curves of type III the first and second phases are similar to those in types I and II. During the third phase there is a definite abrupt increase in the non-acid fluid but the increase in acid fluid is relatively the same as the increase in the non-acid fluid, in consequence of which the acid chloride concentration of the total fluid does not decrease but remains at the same high level that it reached during the second phase, thus producing a plateau. The acid chloride concentration does not rise above 400 mgm. per 100 cc. The amount and time of appearance of the bile are the same as in types I and II.

Curves of type IV are similar to type III except that the non-acid fluid is relatively less in amount. In consequence of this the acid chloride concentration of the total fluid is maintained at a higher level, being 500 mgm. per 100 cc. or higher.

In curves of type V the first and second phases may not be unusual. During the third phase, however, there is a complete disappearance of the non-acid fluid so that all of the fluid entering the stomach appears to be acid fluid. The amount of bile in the samples may be slightly less than in the other types or it may be normal.

The interpretation of the behavior of the non-acid fluid in curves of types I and II offers little difficulty. In studies on whole stomach pouches reported in previous papers it was shown that the non-acid fluid of intragastric origin is quite small in amount and that the secretion rate is fairly constant, showing very little fluctuation. Using an acid test meal, which calls forth a maximum secretion of non-acid fluid of intragastric origin, the average rate of secretion was 6 to 8 cc. per half-hour, this rate being maintained fairly constant for as long as two and one-half hours, marked sudden increases being conspicuously absent. During the first and second phases in curves of types I and II the non-acid fluid is probably mostly of intragastric origin and since the rate of secretion of the acid fluid increases very rapidly during the second phase, the non-acid fluid, which is being secreted at a constant and slower rate, is relatively decreased by dilution with the abundant acid fluid. During the third phase just before emptying occurs the sudden and marked increase in the non-acid fluid is undoubtedly due to regurgitated duodenal secretions.

The disappearance of non-acid fluid during the third phase in curves of type V is difficult to explain. The most obvious explanation might appear to be that there was simply a failure of the normal regurgitation of duodenal secretions. However, this explanation appears inadequate in view of two facts. First, bile was always present in these last samples, often in as great a concentration as in curves of type I and II, and second, the total and the acid chloride concentration of the total fluid entering the stomach was often considerably above 600 mgm. per 100 cc. which should be the maximum value if only acid fluid and no non-acid fluid was entering the stomach. In view of these facts it appears quite likely that curves of type V are not due solely to a diminished regurgitation from the duodenum but also to absorption of water, but not of acid, from the gastric contents just as was found in the whole stomach pouches shown in table 1. Curves of type V were more common when the emptying time of the stomach was prolonged than when it was short, thus giving more time for absorption to occur. Curves of type IV and possibly of type III are probably the result of the absorption of less water than in type V and should therefore be considered as intermediates between the definite types I, II, and V. It is interesting and important to note that when a sufficient number of experiments were done on any of the animals studied it was often possible to obtain all types of curves on the same dog. In figure 1 illustrations of types I, IV and V are from experiments on the same dog. However, curves of types IV and V seem to be much more common in some animals than in others.

3. *The chloride concentration of the fluid entering the stomach:* a. *The total chloride concentration* of the food entering the stomach is goverened by the relative amounts of acid fluid, the higher the per cent of acid fluid, the

higher the total chloride concentration. As shown in figure 2, if only non-acid fluid entered the stomach the total chloride concentration would average 350 mgm. per 100 cc. This is in good agreement with the results of previous studies in which it was found that the neutral chloride concentra-

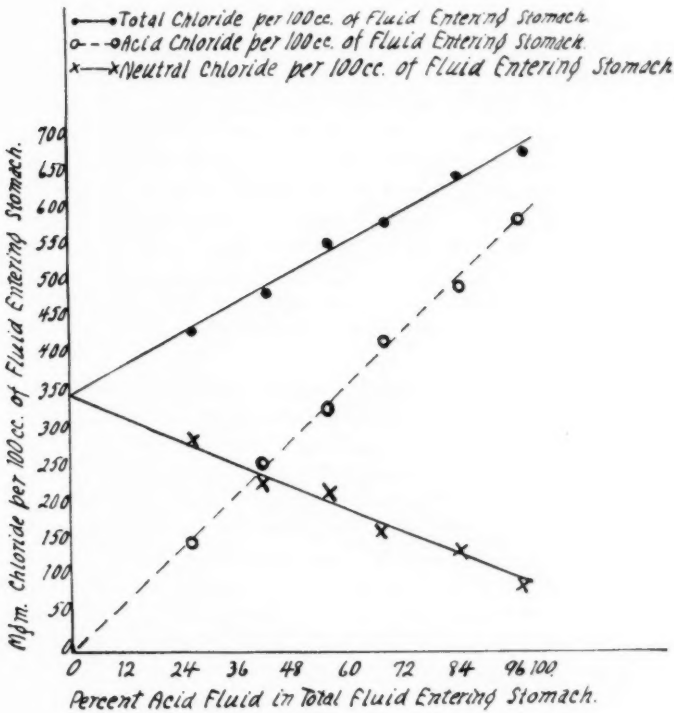

Fig. 2. An analysis of the secretory curves on normal dogs. The samples were grouped according to increasing percentage of acid fluid in the total fluid entering the stomach and the average values for each group plotted. When no acid fluid is present the total chloride concentration would be the neutral chloride present in the non-acid fluid (350 mgm. per 100 cc.). Values for the total chloride in excess of 600 mgm. per 100 cc. indicate absorption of water. The fact that the values in excess of 600 mgm. fall on the same straight line as the lower values suggests that absorption of water is occurring at all times, its effect usually being masked by the entrance of large amounts of non-acid fluid.

tion of the duodenal secretions and of the non-acid secretions of intragastric origin were approximately the same and averaged between 300 and 378 mgm. per 100 cc. Since the pure acid secretion contains approximately 600 mgm. of acid chloride per 100 cc. this should be the highest total chloride

value encountered. The frequent finding of values definitely in excess of this value is explained by the absorption of water from the gastric contents.

b. *The acid and neutral chloride* concentration of the fluid entering the stomach showed, in general, an inverse relationship which was most marked in curves of type I and II and often absent in curves of type V. This inverse relationship is clearly shown in figure 2.

c. *The neutral chloride-extra fluid ratio.* In curves of types I and II this ratio was approximately the same as was previously found in studies with the acid test meal. This is important in showing that the composition of the acid and non-acid fluids in these studies with the Liebig extract test meal is the same as in previous studies with the acid test meal. The average value in 49 samples in which there was no evidence of absorption of water was 4.8 (table 2). If the neutral chloride concentration of the non-acid fluid averages 350 mgm. per 100 cc. (fig. 2) then it can be calculated that the alkalinity of the non-acid fluid averages 0.037 normal (1.3 mgm. of neutralized acid chloride per cc. of non-acid fluid) which is in agreement with the value previously determined (0.04 normal) by direct analysis.

When there is definite evidence of absorption of water from the stomach (types IV and V) the neutral chloride extra fluid ratio rises and may reach very high values (table 2) which indicates that the fluid which is absorbed is relatively poor in neutral chloride.

4. *Bile in the gastric contents.* Some bile was present in the gastric samples in 98 per cent of the 67 experiments. In one experiment (2 per cent) it was entirely absent while in 7 (10 per cent) the amount was not more than a trace. Usually it was absent during the first phase, variable during the second phase and was nearly always present during the third phase. Largely due to water absorption the amount of bile was not a satisfactory index of the amount of non-acid fluid in the sample.

Discussion. A comparison of the experiments on whole stomach pouches with curves of types I, II, and III, (which constitute 92 per cent of the experiments on the intact normal stomach) shows quite clearly that the regurgitation of non-acid fluid from the duodenum is a normal event. In evaluating the importance of duodenal regurgitation much confusion will be avoided if it is remembered that *the primary effect of the regurgitated duodenal secretions is to keep the average acid chloride concentration of the total fluid secretions entering the stomach below the value found in whole stomach pouches.* This is accomplished primarily by dilution and secondarily by neutralization. A comparison of the average acid chloride concentration of the total fluid entering the stomach as shown in tables 1 and 2 illustrates this point. Most investigators have ignored this and have sought for a lowering of the acidity of the gastric contents instead. If the secretion rate of acid is low during the second phase of the secretory curve

TABLE 2

Fractional experiments on six normal dogs showing the different types of secretory curves as illustrated in figure 1

DOG	COMPOSITION OF TEST MEAL		GASTRIC SAMPLE: Total chloride	GASTRIC SAMPLE: Neutral chloride	GASTRIC SAMPLE: P.S.P.	EXTRA TOTAL CHLORIDE	EXTRA NEUTRAL CHLORIDE	EXTRA ACID CHLORIDE	CHLORIDE CONCENTRATION OF GASTRIC SECRETION: Total	CHLORIDE CONCENTRATION OF GASTRIC SECRETION: Neutral	CHLORIDE CONCENTRATION OF GASTRIC SECRETION: Acid	TOTAL FLUID	ACID FLUID	EXTRA FLUID	NEUTRAL CHLORIDE / EXTRA FLUID	BILE	TIME	TYPE CURVE
		mg per 100 cc.	*mg. per 100 cc.*	*mg per 100 cc.*	*cent*												*hrs*	
I	Total	213	249	174	86	66	17	49	471	121	350	14	8	6	2 8	0	¼	I
	Neutral	182	301	153	76	139	15	124	579	63	516	24	21	3	5 0	0	1	
	Acid	31	282	158	46	284	74	210	526	137	389	54	35	19	3 9	+	1½	
I	Total	209	245	168	89	59	16	43	536	145	391	11	7	4	4 0	0	¼	I
	Neutral	171	300	161	75	143	33	110	572	132	440	25	18	7	4 7	+	1	
	Acid	38	360	174	52	251	85	166	525	177	348	48	28	20	4 3	++	1½	
II	Total	222	254	186	91	52	17	35	577	189	388	9	6	3	5 6	trace	¼	I
	Neutral	186	342	156	66	195	33	162	574	97	477	34	27	7	4 7	+	1	
	Acid	36	395	153	50	284	60	224	568	120	448	50	37	13	4 6	+	1½	
			426	180	36	346	113	233	540	177	363	64	39	25	4 5	++	2	
II	Total	215	233	182	91	37	16	21	411	178	233	9	4	5	3 2	0	¼	I
	Neutral	182	291	164	78	123	22	101	559	100	459	22	17	5	4 4	+	1	
	Acid	33	384	146	52	272	51	221	566	106	460	48	37	11	4 6	++	1½	
			408	226	40	322	153	169	537	255	282	60	28	32	4 8	++++	2	
III	Total	226	246	188	94	34	16	18	565	266	299	6	3	3	5 3	0	¼	I
	Neutral	183	294	173	82	109	23	86	605	128	477	18	14	4	5 7	0	1	
	Acid	43	336	165	67	185	42	143	560	127	433	33	24	9	4 7	+	1½	
			362	162	52	245	67	178	510	139	371	48	30	18	3 7	++	2	
			402	192	32	330	133	197	486	195	291	68	33	35	3 8	+++	2½	
III	Total	219	249	183	91	50	23	27	555	255	300	9	5	4	5 7	0	¼	I
	Neutral	176	294	183	82	114	39	75	633	216	417	18	12	6	6 5	++	1	
	Acid	43	344	173	68	195	53	142	609	166	443	32	24	8	6 6	++++	1½	
			432	196	50	322	108	214	644	216	428	50	36	14	7 7	+++	2	
			452	232	36	373	169	204	580	264	316	64	34	30	5 6	++++	2½	
IV	Total	224	251	195	91	47	23	24	523	256	267	9	4	5	4 6	0	¼	I
	Neutral	189	276	197	86	83	34	49	593	243	350	14	8	6	5 7	+	1	
	Acid	35	314	219	74	148	79	69	570	304	266	26	12	14	5 6	++	1½	
			364	210	42	270	131	139	465	226	239	58	23	35	3 7	++++	2	
IV	Total	220	236	191	93	32	23	9	457	329	128	7	2	5	4 6	0	¼	IV
	Neutral	180	266	174	86	77	19	58	550	136	414	14	10	4	4 7	+	1	
	Acid	40	294	182	81	116	36	80	612	190	422	19	13	6	6 0*	++	1½	
			336	186	75	171	51	120	685	204	485	25	20	5	10 2*	+++	2	
			416	174	52	302	80	222	629	166	463	48	37	11	7 3*	+++	2½	
V	Total	220	249	182	91	49	18	31	545	200	345	9	5	4	4 5	0	¼	IV
	Neutral	180	290	168	79	116	26	90	553	124	429	21	15	6	4 3	0	1	
	Acid	40	336	165	71	180	37	143	621	128	493	29	24	5	7 4	+	1½	
			392	178	62	256	66	190	673	174	499	38	32	6	11 0*	++	2	
			464	174	44	367	95	272	655	170	485	56	45	11	8 6*	++	2½	

* Not included in average.

TABLE 2—*Concluded*

DOG	COMPOSITION OF TEST MEAL		GASTRIC SAMPLE: Total chloride	GASTRIC SAMPLE: Neutral chloride	GASTRIC SAMPLE: P.S.P.	EXTRA TOTAL CHLORIDE	EXTRA NEUTRAL CHLORIDE	EXTRA ACID CHLORIDE	CHLORIDE CONCENTRATION OF GASTRIC SECRETION: Total	CHLORIDE CONCENTRATION OF GASTRIC SECRETION: Neutral	CHLORIDE CONCENTRATION OF GASTRIC SECRETION: Acid	TOTAL FLUID	ACID FLUID	EXTRA FLUID	NEUTRAL CHLORIDE / EXTRA FLUID	BILE	TIME	TYPE CURVE
		mg. per 100 cc.	*mg per 100 cc.*	*mg per 100 cc.*	*per cent*												*hrs.*	
V	Total	223	245	179	93	37	8	29	527	114	413*	7	5	2	4.0	0	½	V
	Neutral	184	284	168	83	99	15	84	583	88	495*	17	14	3	5.0	0	1	
	Acid	39	318	155	77	146	13	133	634	57	577*	23	22	1	13.0*	0	1½	
			362	152	70	206	23	183	687	77	610*	30	30	0		trace	2	
			404	146	62	266	32	234	700	84	616*	38	39	0		+	2½	
VI	Total	219	231	177	89	36	20	16	328	182	146	11	3	8	2.5	0	½	I
	Neutral	176	288	182	72	130	55	75	465	197	268	28	13	15	3.7	++	1	
	Acid	43	368	214	28	307	165	142	426	229	197	72	24	48	3.4	++	1½	
VI	Total	219	246	186	86	57	33	24	407	236	171	14	4	10	3.3	trace	½	I
	Neutral	178	312	186	74	150	54	96	578	208	370	26	16	10	5.4	++	1	
	Acid	41	384	266	66	239	148	91	703	436	267	34	15	19	7.8	++++	1½	
III	Total	219	237	177	91	38	15	23	422	167	255	9	4	5	3.0	0	½	II
	Neutral	178	294	183	82	114	37	77	634	206	428	18	13	5	7.4	+	1	
	Acid	41	342	177	66	197	60	137	580	177	403	34	23	11	5.5	++	1½	
			396	168	44	301	90	211	538	161	377	56	35	21	4.3	++	2	
			476	170	36	397	106	291	621	166	455	64	49	15	7.1	++	2½	
VI	Total	227	254	191	91	47	24	23	522	267	255	9	4	5	4.8	0	½	III
	Neutral	184	348	174	52	230	78	152	479	163	316	48	25	23	3.4	+	1	
	Acid	43	420	173	28	356	121	235	494	168	326	72	39	31	3.9	++	1½	
									Average 52 samples, 362						49 samples, 4.8			

then the acidity of the gastric contents will be low and the regurgitated duodenal secretions may be sufficient in amount to lower the acidity of the gastric contents. However, with a powerful and sustained secretagogue such as Liebig's extract, the secretion rate during the second phase is very high, hence the acidity of the gastric contents is high and the regurgitated duodenal secretions may not be able to bring about an actual lowering; *the effect being rather to cause a less rapid rise or to prevent further rise.* Examination of column 8 (extra acid chloride in the gastric content), table 2, illustrates this point. If these distinctions are borne in mind much of the confusion centering around the question of duodenal regurgitation will be avoided.

Absorption of water from the gastric contents as a factor in offsetting the effect of duodenal regurgitation needs further study. It is quite possible that absorption of water is a normal process which is always taking place in the stimulated stomach but that ordinarily sufficient non-acid fluid enters

the stomach to mask the effect so that absorption of water cannot be detected by analysis of the gastric contents. If, however, the amount of non-acid fluid entering the stomach should for any reason be diminished, then absorption would be detected by the finding of an abnormally high acid and total chloride concentration of the total fluid entering the stomach. On the other hand absorption of water may be an irregular phenomenon which may occur in any animal on certain occasions, the frequency of its occurrence differing in different animals.

It is possible that certain types of hyperacidity may be the result of excessive absorption of water from the gastric contents rather than a failure of duodenal regurgitation or excessive secretion of acid. Absorption of water from the gastric contents appears to be more common, or its effect is more pronounced, when the emptying time of the stomach is prolonged and it is probable that the high acidity of the gastric contents in cases of pyloric obstruction may be the result of absorption of water with concentration of the acid rather than of continued secretion. As pointed out above, absorption of water may be related to and possibly controlled by the acidity of the gastric contents since we found no evidence of its occurring when a tenth normal hydrochloric acid test meal was used. Absorption of water with resulting concentration of the acid may also explain a statement frequently encountered in physiological literature dealing with studies on fundic or whole stomach pouches, namely, that the acidity of the secretion often shows a definite increase without a corresponding increase in volume.

We have not found evidence of direct absorption of acid from the stomach. The water which is absorbed from the stomach undoubtedly contains some neutral chloride and it is possible that small amounts of acid may be indirectly absorbed by being neutralized and then absorbed as neutral chloride. However, if this does occur it appears to be a negligible factor in the regulation of gastric acidity.

In those experiments in which there was no evidence of absorption of water, the composition of the various secretions entering the stomach appears to be the same as that previously found in studies with the acid test meal with and without histamine stimulation. The finding of a neutral chloride extra fluid ratio of approximately the same average value lends support to this statement.

SUMMARY

1. The preparation and use of a two per cent Liebig's extract test meal containing 15 mgm. of phenol red per liter is described.

2. The gastric secretory curve in the intact normal stomach is described and compared with that in whole stomach pouches.

3. The results show that regurgitation of duodenal secretions is a normal

event and that the results of regurgitation were clearly evident in 92 per cent of 67 experiments on 11 normal animals. The primary result of the regurgitation of duodenal secretions is to keep the average acid chloride concentration of the total fluid entering the secreting stomach below the average value found in isolated whole stomach pouches. With a powerful and maintained stimulus such as Liebig's extract, the regurgitated duodenal secretions are seldom sufficient in amount to cause an actual lowering of the acidity of the gastric contents the result being rather to prevent further rise or to cause a slower rise.

4. Absorption of water from the gastric contents, with concentration of the acid, appears to be a factor of considerable importance in offsetting the effects of duodenal regurgitation and producing irregularities of the gastric secretory curve.

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ON THE RATE OF SECRETION OF BILE

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Since 1889 when Copeman and Winston (1) reported their studies on the bile secreted by an apparently healthy patient with a biliary fistula, there have been several conflicting reports on the rates of bile secretion during the 24 hours. Josephson and Larsson (2) in discussing the literature up to 1933 note that of all previous studies, only those of Copeman and Winston (1), Robson (3) and Pfaff and Balch (4) were made on patients who were not at the same time suffering from some severe disturbance of liver metabolism.

Copeman and Winston (1) studied the rate of bile secretion at hourly intervals for 24 hours. They found maxima of secretory activity at 12 noon and 12 midnight though their patient received five meals a day at 5 a.m., 10 a.m., 1 p.m., 4:30 p.m. and 7:30 p.m., the largest meal being at 1 p.m. The minimum of secretory activity occurred at 5 a.m.

Robson's (3) case was studied for 48 hours. Although the two 24-hour periods differed very widely from each other, it is clear from their data that the rate of secretion was greater during the waking hours.

Pfaff and Balch's (4) study covered 60 consecutive hours. Their maxima occurred at 12 noon and 2 p.m. and a minimum during the early morning hours.

The inadequacy of the data given by previous observers became more marked when Forsgren (5) (1928) reported that histologic studies on the livers of rabbits killed at various times during the 24 hours showed that the secretory activity was at a minimum in the early morning hours and at a maximum at some time later in the day.

Tremolieres, Thiéry and Fauchet (6) studied three hour intervals for eight days. They also found a much lower rate of secretion during the sleeping hours and a maximum in the three hours ending at 2 p.m.

It was to obtain more definite data on the question of the periodicity of bile secretion that Josephson and Larsson (2) studied their patient for 5 consecutive days. It is clear from their data that the rate of secretion is greater during the waking hours, the minimum occurring in the two hour interval ending at 5 a.m. and the maximum in the two hour interval ending at 3 p.m. Their data were scattered so widely, however, that they were

unwilling to draw any specific conclusions concerning periodicity in the rate of bile secretion.

The patient we observed had had two previous operations for cholelithiasis, a cholecystectomy and a choledochotomy. One year after her second operation, she presented herself with a history of biliary colic and jaundice. At operation, the common duct was found to be dilated to a diameter of 1½ cm., and about 60 calculi, most of which were between ½ and 1 cm. in diameter, were removed from the common duct and both hepatic ducts well up in the liver. A large T tube was snugly fitted into the common duct, sutured in place and the bile allowed to drain into a bottle. She convalesced uneventfully, her stools remaining free of bile as long as the tube was left open. She was kept under observation continuously on a low fat diet for the following nine months up to the present. At this time she still has the T tube so well fitted that as long as the tube remains open, the intestine is free of bile.

Since the validity of our observations depended on the knowledge that all the bile would flow out through the unobstructed T tube, we also determined the resistance of the sphincter of Oddi in terms of the amount of pressure necessary to produce flow of liquid into the duodenum. This was done by observing the height to which a column of water could be raised above the end of the common duct without any loss into the intestine. The end of the common duct had been previously determined by allowing a column of thorotrast to flow into the system, and watching its appearance in the duodenum under the fluoroscope with the patient in the upright position. It was found that the vertical height from the point where the tube entered the abdomen to the end of the common duct was 7 cm. and that an additional height of 16 cm. was necessary for water to overcome the resistance of the sphincter. This total pressure of 23 cm. of water, although a low one, was undoubtedly a safe one for this experiment.

One week after operation, after our patient had run a normal temperature for three days, we began to study the rate of bile secretion. The bile was allowed to flow into a graduated cylinder and observations were made as nearly as possible at two hour intervals. Occasionally the observation was made at an odd time which was recorded. From these determinations a smooth curve was plotted for each day, and wherever the two hourly observation was missing, the amount of secretion was read from the curve.

Figure 1 shows the amount of bile secreted in each two hour interval for part of the experiment as calculated from these observations. At first glance the only regularity to be noted in this graph is the definitely dimin-

ished rate of secretion between 9 p.m. and 6 a.m. which roughly correspond with the patient's sleeping hours. The average rate of secretion during the waking hours is 23.4 cc. per hour, while the average rate during the sleeping hours is 15.9 cc. per hour.

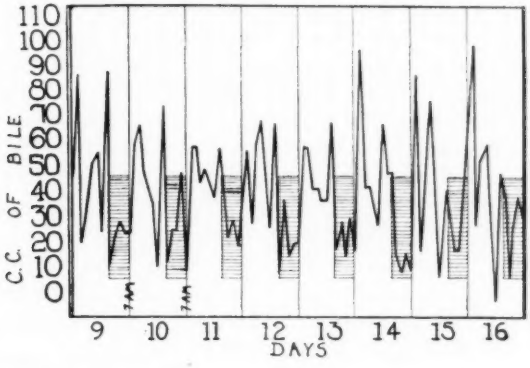

Fig. 1. Volume of bile in cubic centimeters per two hours for 8 days. The shaded areas represent sleeping hours.

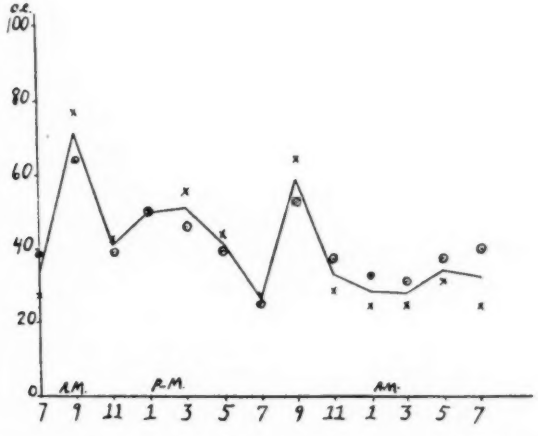

Fig. 2. Average secretion for 2-hour intervals. —— Average for 16 days. ⊙ Average for first 8 days. × Average for second 8 days.

To study these data in more detail a sixteen day average was computed for each two hour interval and a composite curve plotted (fig. 2). The peaks in the curve are so definite that it was thought worth while to compute the average values for the two eight day periods separately. These values, indicated in figure 2, follow the total average very closely. It is interesting to note that the maxima in the rate of bile secretion occurred in the two hour interval immediately after breakfast, and in the two hour interval beginning two hours after supper. Since the lowest rate of secretion during the waking hours occurred in the two hour period immediately following supper and since the noon meal is not related to any significant change in rate, it would seem that the periodicity in the rate of secretion of bile is not simply related to the ingestion of food.

The volume of bile secreted daily was fairly constant during the whole experiment. For the first eight days, the average was 495 $\pm$ 20 cc. ranging from 420 cc. to 540 cc. For the second eight days the average was 491 $\pm$ 7 cc. ranging from 480 cc. to 510 cc. The only explanation we can offer for the greater regularity during the second eight days is that the patient was further advanced in her convalescence.

SUMMARY

1. In a healthy patient with a bile fistula, the rate of bile secretion was studied for sixteen consecutive days.

2. The rate of secretion was found to be greater in the waking hours than in the sleeping hours.

3. The composite values for the rates of secretion give a curve with two distinct maxima. There was no regular increased secretory activity following the ingestion of food.

4. The secretory activity was more regular during the second eight days of study than during the first.

5. The sphincter of Oddi was found to withstand a pressure up to 23 cm. of water before allowing the passage of fluid into the duodenum.

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THE PHENOLSULPHONEPHTHALEIN RENAL FUNCTION TEST IN DOGS[1]

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An improved procedure for performing the phenolsulphonephthalein renal function test in unanesthetized dogs has been devised. Since the literature (1), (2), (3), (4), (5), (6), (7) contains only incidental or otherwise unsatisfactory inclusions of data obtained on normal dogs, this test has been applied to a number of such animals.

TECHNIQUE. 1. About 10 cc. of water per kgm. are administered by means of a stomach tube.

2. About 15 minutes later the bladder is emptied by means of a catheter, and the urine is discarded.

3. The dog is held in a comfortable position for the remainder of the test to prevent urination.

4. By means of a tuberculin syringe, ½ cc. of a properly prepared phenolsulphonephthalein solution, of such strength that 1 cc. contains 6 mgm. of the dye, is injected intravenously. (Hynson, Westcott, and Dunning put up such a solution in ampules.)

5. The urine is collected by catheterization 1 hour after the injection of the phenolsulphonephthalein. *The bladder is washed out with about 100 cc. of 0.9 per cent sodium chloride solution to insure complete recovery of the dye.*

6. Dilution of the urine sample: The urine is diluted to 500 cc. in a volumetric flask after the addition of 5 cc. of 10 per cent sodium hydroxide solution. *The diluted urine is centrifuged to eliminate turbidity.*

7. Standard: The standard is made by diluting 1 cc. of the phenolsulphonephthalein solution to 1000 cc. after adding 10 cc. of 10 per cent sodium hydroxide.

8. The standard and the diluted urine are compared in a Duboscq colorimeter, placing the color filter described below over the eye-piece.

The color filter. Frequently, because of the color from the dog urine, it is impossible to match the standard and the diluted urine. This difficulty may be overcome by using a color filter which absorbs light of the same wave lengths as does the urine. A filter, made by the Corning Glass Works, H.R. lantern yellow, no. 349, 2 cm. in diameter and 3.5 mm. in thickness, is satisfactory, and was employed in the present work.

In order to select this filter, the absorption spectra of phenolsulphone-

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

phthalein and of several dog urines were determined by means of a spectrophotometer. Samples of the data are presented in the figure. As will be seen from the curves, the filter shuts out light of the wave lengths mainly absorbed by dog urine and yet transmits some of the light most strongly absorbed by phenolsulphonephthalein.

The filter permits colorimetric comparison of the phenolsulphonephthalein standard and the diluted urine sample without any great interference on the part of the urinary pigments.

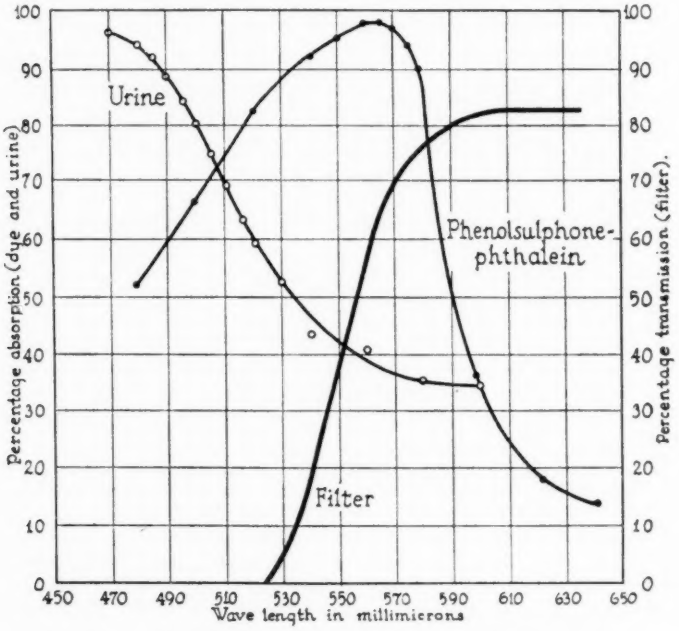

Fig. 1. Absorption spectra of dog urine, alkaline phenolsulphonephthalein solution, and Corning filter, H. R. lantern yellow, no. 349.

Experiments, in which equal amounts of distilled water and dog urine were added to the same volume of the standard phenolsulphonephthalein solution, bear out this contention. With the color filter, the colorimeter showed the solutions to have approximately (within 5 per cent) the same concentration of dye. Representative samples of such experiments are given below. In the first experiment the proportion and concentration of urine were adjusted to approximate the conditions found in a renal function test on a dog; in the other experiment the proportion of urine was made greater.

Experiment 1. Water solution: Fifty cubic centimeters of standard phenolsulphonephthalein solution (6 mgm. of the dye per liter with 10 cc. of 10 per cent sodium hydroxide). Ten cubic centimeters of water.

Urine solution: Fifty cubic centimeters of standard phenolsulphonephthalein solution. Ten cubic centimeters of dog urine. This solution was *centrifuged* to avoid turbidity.

TABLE 1

Phenolsulphonephthalein excretion for a 1 hour period in normal, adult male dogs

DOG	DATE	INDIVIDUAL DETERMINATIONS	AVERAGES FOR INDIVIDUAL DOGS	DOG	DATE	INDIVIDUAL DETERMINATIONS	AVERAGES FOR INDIVIDUAL DOGS
		per cent	*per cent*			*per cent*	*per cent*
1	5-22-35	70		7	6-27	70	
	5-24	63			7-1	65	
	5-25	61			7-3	75	
	5-27	60	64		7-12	73	71
2	5-22	77		8	6-27	84	
	5-24	67			7-2	85	
	5-25	78	74		7-5	80	83
3	5-22	72	72	9	7-5	77	
					7-8	72	
4	5-24	68			7-15	71	73
	6-1	64					
	6-8	66		10	7-23	74	
	6-17	69	67		8-6	74	74
5	5-24	61		11	7-30	78	
	5-25	69			8-6	80	79
	5-27	72					
	6-1	65	67	12	8-1	80	
					8-6	81	
6	6-24	66			8-14	70	77
	6-27	70					
	7-1	67		13	7-30	78	
	7-3	70	68		8-6	80	
					8-14	69	76

By colorimetry, using the color filter, the concentration of dye in the urine solution was found to be 103 per cent of that in the water solution. Thus there is a 3 per cent error.

Experiment 2. Water solution: Forty cubic centimeters of standard phenolsulphonephthalein solution. Twenty cubic centimeters of water.

Urine solution: Forty cubic centimeters of standard phenolsulphonephthalein solution. Twenty cubic centimeters of dog urine. *Centrifuged.*

By colorimetry, using the color filter, the concentration of dye in the urine solution was found to be 101 per cent of that in the water solution.

RESULTS ON NORMAL DOGS. Forty determinations are presented on 13 normal, adult male dogs. The average phenolsulphonephthalein excretion for these dogs is 72.7 per cent. The range is from 60 to 85 per cent, but readings on individual animals did not vary more than 11 per cent among themselves. The data are given in the accompanying table.

SUMMARY

1. The technique for the phenolsulphonephthalein renal function test in dogs is presented. A color filter is employed in the colorimetric comparison, in order to correct for the color of the urinary pigments.

2. Forty determinations on 13 normal, adult male dogs are given. The average excretion for a 1 hour period with intravenous injection is 72.7 per cent with a range of 60 to 85 per cent.

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PROPERTIES OF RED CELL SURFACES INFLUENCING ROULEAU FORMATION

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We have recently presented evidence (Monaghan and White, 1936) that rouleau formation, the intensity of which is the principal factor determining sedimentation velocity of red cells, does not involve an adsorption of proteins by the cell surface and that proteins increasing the sedimentation velocity of a given sample of cells act by abstracting oriented surface water, thereby increasing the interfacial energy at the cell-medium interface and thus increasing the probability of cohesion of colliding cells. The paper referred to was concerned primarily with a comparison of the rouleau-promoting activities of various proteins but it also was pointed out that cells of different species react differently to a given protein. The present paper reports on an investigation of the extent to which differences inherent in the cells of different species influence rouleau formation in a given medium and gives some information as to the nature of these differences.

Five types of observations were made; 1, determinations of the electrophoretic mobilities of normal red cells at pH 7.4; 2, determinations of the sedimentation velocities of cells in 1 per cent gelatin at pH 7.4; 3, microscopic observations of the extent of rouleau formation, 4; determinations of the isoelectric points of washed red cell ghosts, and 5, determinations of the extractable lipoid content of stroma material.

The fact that one species of cells undergoes greater aggregation, as shown by increased sedimentation velocity and by microscopic observation, in a given medium than does another species is *prima facie* evidence that the sum total of factors contributing to surface energy per unit area of cell-medium interface is greater in the case of the first species. The following factors may be considered. The inherent surface energy at the cell-medium interface, aside from any part played by surface solvation or electrical forces, may be greater with the aggregating species. On the basis of considerations discussed by Mueller (1933) it appears probable, however, that in every case aggregation should result with particles as large as red cells, provided no additional factor or factors were operative in reducing surface energy. In spite of the theoretical prediction of instabil-

ity (where surface solvation and electrical forces are left out of account), the fact is that all species of mammalian red cells are stable, i.e., do not aggregate into rouleaux, in salt solution. It therefore follows, granting the validity of the calculations of Mueller (1933), of March (1927), and of Gyemant (1935), that other factors, as surface hydration or electrical forces or both, reduce the interfacial energy to a level compatible with stability in salt solution. It thus becomes desirable to investigate the interfacial or electrophoretic potentials of the various species of cells. Further consideration of the matter will be taken up later in this paper.

METHODS. For a mobility determination at pH 7.4 a drop of oxalated blood is put into about 50 cc. of M/50 phosphate buffer of pH 7.4 plus 0.3 per cent sodium chloride plus 6 per cent sucrose and the mobility determined in the cylindrical cell according to the technique previously described (Monaghan and White, 1936). Sucrose is substituted for part of the salt to avoid too high a current density in the cell.

Sedimentation velocities were determined in 1 per cent gelatin plus 0.9 per cent sodium chloride plus M/50 phosphate buffer at pH 7.4, using the technique previously described (Monaghan and White, 1936). Readings were made at 20 minutes. In comparing the sedimentation velocities of cells of various species in a given medium the determinations must all be carried out on the same day with fresh blood, since the properties of both cells and medium concerned in rouleau formation may change within a day or two.

In determinations of the isoelectric points of the red cell ghosts a sample of oxalated blood is centrifuged and the cells washed five times in at least five times the cell volume of 0.9 per cent sodium chloride. The washed cells are laked by adding 9 volumes of distilled water and the remaining white cells centrifuged down; the ghosts are not thrown down with speeds up to 2500 r.p.m. The laked cells are then acidified by slow addition of N/10 HCl (about 5 cc. to 50 cc. of the 1–10 laked cells) until the stromata are precipitated at about pH 5.5. They are then centrifuged down and washed several times with distilled water until hemoglobin is no longer present in the washings. The ghost residue still contains some hemoglobin which is removed by the following procedure. To the residue from 5 cc. original cell volume are added about 25 cc. N/100 NaOH and 10 cc. toluol, the suspension is then shaken vigorously and centrifuged. The ghosts come to the aqueous-toluol interface, the bottom aqueous layer is pipetted off, a fresh 25 cc. of N/10 NaOH added and the process repeated twice. Some of the ghosts are lost with each washing, since the separation is not complete. A final washing is carried out with distilled water. The clear toluol at the top is finally pipetted off and the remaining toluol blown off in an air current. The resultant suspension shows nothing in the bright field but on dark field examination is seen to consist in part of

ghosts which retain their individuality as cells and in part of fragments. Suspensions of this material are made up in HCl-KCl or NaOH-KCl mixtures of M/50 concentration and varying pH and the pH-mobility curve determined, pH of each suspension after the addition of the ghosts being measured with the glass electrode.

The method used for preparation of stroma material is a combination of the procedures of Jorpes (1932) and of Haurowitz and Sladek (1928) with the modification that the precipitate is washed with N/100 NaOH instead of with water or 0.9 per cent NaCl. We found that after the washings of water or 0.9 per cent NaCl had become clear the stroma material still contained considerable hemoglobin, which could be removed by washing with N/100 NaOH.

Jorpes (1932) obtained a precipitate from washed laked cells at pH 5.5 and assumed that this is the isoelectric point of the ghost protein and also

TABLE 1

Summary of sedimentation and mobility findings

SPECIES	MOBILITY OF NORMAL CELLS AT pH 7.4	ISOELECTRIC POINT OF WASHED GHOSTS	SEDIMENTATION VELOCITY IN 20 MINUTES
	μ/sec./vl./cm.		*mm.*
Dog	1.59	2.7	122
Horse	1.56		111
Human	1.34		94
Cat	1.34		57
Rat	1.31		46
Pig	1.02		36
Cow	0.98	3.4	2
Rabbit	0.47	4.3	0

of the ghosts themselves. He found, however, that from 14 to 30 per cent of this precipitate, even after repeated washing in a buffer of pH 5.5, was hemoglobin.[1] We believe that when a laked cell suspension is brought to pH 5.5 a precipitate is formed because the hemoglobin is now sufficiently positively charged to be adsorbed by the negatively charged ghosts with resultant mutual precipitation. The isoelectric point of the hemoglobin-free ghosts is, as we find in this paper, between 2.7 and 4.3, depending on species. Jorpes did not determine the isoelectric point of his relatively hemoglobin-free stroma material.

Table 1 gives the sedimentation velocities of cells in 1 per cent gelatin, the electrophoretic mobilities of cells in sucrose-salt-buffer at pH 7.4 and

[1] In view of Barkan's (1927) findings on the non-hemoglobin iron content of red cells and ghosts these figures do not have the quantitative significance attributed to them by Jorpes but it is certainly true that some hemoglobin was still present.

the isoelectric points of washed ghosts. It is seen that the greater the sinking velocity the greater the electrophoretic mobility at pH 7.4 and the lower the isoelectric pH of the corresponding ghosts. In all cases microscopic examination confirmed that high sinking velocity was a manifestation of highly developed rouleau formation.

A finding not shown in the table is that the mobility of the washed ghosts at pH 7.4 is the same as that of the intact cells of the same species, indicating that the essential properties of the surface have not been greatly altered.

An attempt was made to determine the proportion of lipoid to protein in the stroma material of different species by extraction with various fat solvents. Dog, cow and rabbit stroma preparations were dried in an oven at 80° and extracted with cold alcohol and ether. The alcohol and ether fractions were combined, dried and weighed. This fraction constituted for the dog 62 per cent of the dry weight of the original stroma material, for both the cow and the rabbit 51 per cent. The isoelectric pH of the remaining "protein" fractions were: dog, 3.3; cow, 3.4, and rabbit, 5.1. It was found, however, that when the supposed protein material was suspended in dilute NaOH and shaken with toluol, all of the "protein" came into the toluol phase; obviously considerable amounts of lipoid had not been extracted by the cold alcohol and ether. Continuous 3 hour extraction with hot alcohol and ether removed 71 per cent of the dry weight of dog ghosts and 53 per cent of cow ghosts but again the remaining material came to the toluol phase in a mixture of toluol and water. A very prolonged extraction of dried dog ghosts with boiling alcohol, ether, chloroform and toluol (1 day each) freed the protein of fat sufficiently that it remained suspended in the water on shaking with toluol, but because of the difficulty of obtaining large amounts of hemoglobin-free material for analysis and the uncertainty as to complete removal of the fat further analyses were not made. The only positive conclusion to be drawn from this work is that at least part of the lipoid material must be quite firmly bound to the protein. Evidence on the basis of these determinations that the lipoid/protein ratio is higher with the rapidly sinking cells is suggestive but not conclusive.

Discussion. We are interested in this paper in two distinct but interdependent questions: 1, the mechanisms which determine differences in the capacities of various red cells to form rouleaux, and 2, the nature of the normal red cell surface. As regards the first question, it is evident that stability is not proportional to zeta potential, rather the reverse is true. Greater aggregation with higher zeta means that zeta, while it may give an indication of the surface properties, is not in itself an important factor in determining stability by the conventional mechanism of electrostatic repulsion.

The probability that surface hydration or electrical forces or both operate to lower cell-medium interfacial energy has already been mentioned. Surface charge is to be thought of as operating in two ways to lower interfacial energy: 1, the well known effect of an electrical charge in lowering surface tension, and 2, the action of electrostriction (Mueller, 1933, 1935) set up by the electrokinetic potential, in forming an oriented water layer around the particle. In addition to these processes, electrostatic repulsion due to the electrical double layer must play some part in determining stability. All three of these factors, in so far as they are operative, will work in such a way that an increase in electrokinetic potential favors increased stability. It might therefore be supposed that the species of cells showing the highest potentials should be most stable in salt solution. The question cannot be answered since all species of mammalian red cells investigated are permanently stable, i.e., do not exhibit any rouleau formation, in salt solution. When, however, the various types of cells are suspended in 1 per cent gelatin great differences in stability are seen, as the data show. But the results are consistently the reverse of what might be predicted from the points discussed earlier in this paragraph, i.e., stability varies in an inverse sense with electrokinetic potential.[2]

It therefore appears that at blood pH the electrical factors play a minor rôle and some other surface property predominates in determining stability in gelatin. The mobility and isoelectric point determinations indicate that this predominant factor is the extent of surface hydration. The red cell membrane is known to contain both protein and lipoid material (Jorpes, 1932; Haurowitz and Sladek, 1928; Beumer and Bürger, 1912). The low isoelectric point of ghosts indicates that the membrane surface is made up of lipo-protein.[3] Further evidence of such a lipo-protein surface is given by the interfacial technique experiments of Mudd and Mudd (1931), as well as by the simple observation that red cells or ghosts come to a toluol-water interface on being shaken in a test tube. In view of the low isoelectric points of the lipids known to occur in red cells one would expect the types of cells having the highest ratio of lipid to protein in their surfaces to have the lowest isoelectric points and to show the greatest mobilities at pH 7.4. One would also expect the high-lipid cell surfaces to be the least able to hold water, which condition will increase surface energy and therefore the degree of aggregation. This correlation is borne out by our data, i.e., the cells which are most aggregated show the lowest isoelectric points and the highest mobilities at pH 7.4. The

[2] We have recently shown (Monaghan and White, 1936) that the mobilities of various species of red cells are arranged in the same order in 1 per cent gelatin as in salt solution and are only slightly lower in the former.

[3] The isoelectric point of lecithin is pH 2.6, of cholesterol 3.2.

gelatin presumably acts by abstracting surface water (Monaghan and White, 1936), which process is the more easily carried out the higher the lipid content of the surface. This results in an increased interfacial energy with consequent increased aggregation.

The findings thus indicate that the normal red cell surface is one of lipo-protein and that the lipid content is highest with the cells which aggregate most readily. Further extraction data are required to establish this by direct chemical analysis. It must be emphasized here that it is practically impossible to determine the isoelectric point of an intact red cell, since its surface changes so rapidly in media of low pH (Abramson, 1934). Most observations purporting to show that red cells have a protein coat adsorbed on them may be explained on the basis of failure to recognize this fact.[4] We believe that our observations on the isoelectric points of *washed* ghosts of various species give more nearly the true isoelectric points of normal red cells than have any of the attempts on intact cells.

SUMMARY

1. The sinking velocities and rouleau formations of dog, horse, human, cat, rat, pig, beef and rabbit red cells in 1 per cent gelatin at pH 7.4 have been investigated, also their electrophoretic mobilities at pH 7.4. The isoelectric points of washed dog, beef and rabbit ghosts have been determined electrophoretically. The cells can be arranged in a consistent order, the greater the sinking velocity the greater the mobility at 7.4 and the lower the isoelectric point.

2. Since the cells with the higher mobilities show the greater aggregation, electrostatic repulsion is not the predominant factor in determining stability in 1 per cent gelatin. The findings indicate that the free surface energy at the cell-medium interface is the determining factor. This in turn is determined largely by the degree of surface hydration, which is determined by the chemical structure of the cell surface.

3. The red cell surface probably consists of a combination of lipoid and protein. The mobility data are interpreted as showing that the lipoid/protein proportion varies with different species, being high with dog and horse cells, low with beef and rabbit cells. Further extraction data are required to establish this by direct chemical analysis.

4. The isoelectric points of washed dog, beef and rabbit ghosts are 2.7, 3.4 and 4.3, respectively. These almost certainly are a closer approach to the isoelectric points of intact cells than can be obtained by working with intact cells.

[4] This topic, together with a discussion of the recent work of Bellis and Scott (1935), will be further considered in a subsequent communication.

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O_2 AND CO_2 TENSIONS IN THE SUBCUTANEOUS TISSUES OF NORMAL SUBJECTS[1]

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Our present knowledge concerning the O_2 and CO_2 tensions in the tissues is largely based upon the researches of Campbell (1) who, in reviewing the subject in 1931, presented a critique of methods available for such a study and a summary of previous findings. Studies of the subject to the present time, with but few exceptions, have been made on laboratory animals. This communication deals with a study of the gas depot method (2) in the normal human subject with the object in view of its subsequent application to a clinical analysis of drug action and disease.

METHOD. One hundred cubic centimeters of commercial nitrogen is injected into the subcutaneous region over the anterior abdominal wall. This area was selected in these studies because of the flexibility of the skin and the loose subcutaneous attachment. The simple apparatus used for injection consists of a 50 cc. syringe connected through a three-way stopcock in such a manner that the nitrogen contained in a small rubber bag is aspirated into the syringe, and subsequently filtered through a container filled with cotton to remove any gross particles before entering the tissues. If the gas is slowly injected, the sensation produced is described subjectively as "crawling," "tingling" or "stretching." Distinct pain sensations arise only if the injection is rapid or of such quantity as to cause an excessive stretching of the tissues. Injected into the derma, the gas tends to spread rapidly along the sheaths of the superficial veins, as indicated by Bazett and Sribyatta (3), and is difficult to recover. The ideal pocket lies between the superficial and deep fascia, in which position the gas is easy to recover since it tends to remain *in situ*. Some tenderness to pressure is present for a few days during the period of reaction following the injection. This varies in different individuals and is much less if the deeper pocket is obtained. Because of absorption and spreading of the gas, only small samples can be easily recovered, necessitating a method of sampling which absolutely precludes contamination with air or contact with water, as well as an accurate method of microanalysis. The

[1] Supported by the Wisconsin Alumni Research Foundation.

sampling apparatus, in which mercury is used as the confining liquid, was devised to answer the former requirement. Details of the apparatus will be published elsewhere.

A small amount of clear serum may be drawn into the apparatus without influencing the final results. If blood or blood-tinged serum is obtained, the sample must be discarded, as it will usually affect the final result. Replenishment of gas within the pocket following sampling is accomplished through the needle after detachment from the sampling apparatus. In these experiments, 50 cc. of nitrogen was injected immediately after the daily sampling, it having been determined that this injection does not alter the composition of the next 24-hour sample.

The analysis is accomplished by the excellent method of Blacet and Leighton (4). Their apparatus is made without stopcocks and utilizes dry reagents, these two factors eliminating many of the errors incident to the use of ordinary micromethods. The size of the sample analyzed is about 0.11 cc. so that a 2 cc. sample allows for repeated checks. We have obtained a degree of accuracy with this method of ± 0.1 to 0.3 per cent.

In correcting the readings obtained at analysis, to the tensions actually existing in the tissues, the temperature of the tissues has been considered to be 37°C. The tension of aqueous vapor at 37°C. is 46 mm. Since the partial pressures obtained by the method of analysis are for the dry gas, the following formula has been used to correct the final results to 37°C. and 740 mm. pressure:

$$\text{Volume per cent of dry gas obtained at analysis} \times \frac{\text{Obs. Bar. Pres.} - 46}{100} \times \frac{740}{\text{Obs. Bar. Pres.}}$$

Skin temperatures in this region are usually slightly lower than 37°C. Any error introduced through this source, however, would be a relatively constant and negligible one in the final calculation. Pressure above atmospheric exerted by the tissues on the gas enclosed in the pocket is so small that it has been neglected.

DISCUSSION OF RESULTS. Two representative curves for normal subjects are shown graphically in figure 1. It will be noted from these curves that a period of several days (usually five to seven) is required before a value which represents final equilibrium is obtained. These results support the finding of Campbell in animals that the introduction of a gas into any tissue or body cavity stimulates a foreign body reaction on the part of the cells in immediate contact with gas. A sterile inflammation results which alters the calibre, and possibly the integrity, of the capillaries. Campbell found that after the initial hyperemia, a remarkable constancy obtained in animals from day to day over periods as long as a year, and that subsequent injections of gas into the pocket or region did not elicit any further reaction. The crossing of the curves of the two

gases as the inflammatory reaction subsides, was a constant finding in each of the five normal subjects here reported, and in fifteen patients thus far studied.

The average tension for each gas in the five subjects, after final equilibrium was established, is seen in table 1. Considerable variation is to be noted, as Campbell found to be true for the lower mammals.

The average tension of CO_2 in the tissues of five subjects, after final equilibrium had become established, was 45 mm. of mercury. Figures given in the literature for the CO_2 tension in venous blood vary between 42 and 46 mm. (5) (6) (7). Haldane (8) and subsequent investigators place the alveolar CO_2 tension between 35 and 40 mm. While no determinations of the CO_2 tensions in alveolar air or venous blood were made on

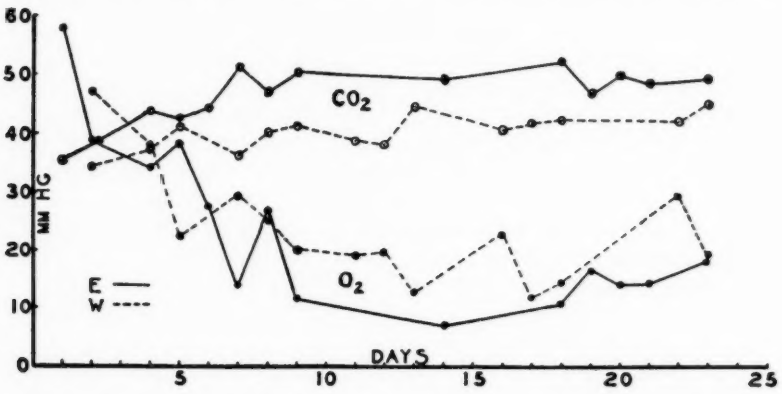

Fig. 1. Curves of O_2 and CO_2 tensions in the subcutaneous tissues of two subjects over a three week period. Initial nitrogen injection at zero. Upper curves, CO_2; lower, O_2. Note the crossing of curves as the inflammatory reaction subsides.

these subjects, a comparison of the present results with those cited indicates that the CO_2 tensions in the tissues studied approximate or exceed those for venous blood.

The average tissue CO_2 value for these five subjects 24 hours following the initial injection of nitrogen, and before a final equilibrium had become established, was only 37 mm. This figure is of the same order as that found by Bazett and Sribyatta (3) who studied the gas tensions for a few hours after injection of nitrogen under the skin of their own forearms. These authors demonstrated that baths at different temperatures exerted a considerable effect upon the tissue tensions in this region. Whether the fact that their studies were made during the inflammatory period influenced the results is difficult to say with the evidence at present available. It might appear that other factors than the physical effects of temperature

play an equally important rôle in view of the results herein presented, since a rise in CO_2 tension as inflammation subsides is a uniform finding in spite of the fact that the local temperature decreases with the subsidence of the inflammatory reaction.

Meyer (9) recently found tensions of 41 mm. of CO_2 and 37 mm. of O_2 under the skin of the thigh in patients at bed rest, four days after the initial injection of nitrogen or air. We have not been able to obtain equilibrium values in the tissues of the abdominal wall after this time period in normal subjects who were not limited in their activity, except for a period immediately preceding sampling. As will be noted in figure 1, the present findings are of the same order as those found by Meyer at the four day post-injection period.

In a general way, a reciprocal relationship appears to hold for the tensions of the two gases after final equilibrium has become established; that

TABLE 1

Average O_2 and CO_2 tensions in the skin of five normal subjects after inflammation had subsided

SUBJECT	AGE	CO_2	O_2
		mm. Hg	*mm.* Hg
R	35	43	24
W	52	41	20
E	22	49	15
P	30	45	27
M	50	50	24
Average		45	22

is, if the O_2 tension is high, the partial pressure of CO_2 is diminished, and vice versa. This probably results from variations in the volume and speed of blood flow to the local area. It has been found in experiments not here reported that factors which alter the O_2 and CO_2 economy of the body as a whole, change this relationship. This is possibly true of local changes in temperature, as is indicated from the curves of Bazett and Sribyatta (3).

It will be observed from the curves in figure 1 that the CO_2 values show much less daily variation in a given individual than those for O_2. Changes in the partial pressure of CO_2 in the tissues, according to Campbell, are of more significance in demonstrating alterations in pulmonary function, while those of O_2 indicate primarily variations in the circulation, although it is obvious that a diminished O_2 saturation of arterial blood would be reflected in the tissue tensions of this gas.

Campbell found the O_2 tensions in the monkey and man to be higher than in other mammals. His observations on man, as he admits, did

not extend over a long enough period to pass the inflammatory stage. The actual values for the tensions existing in the subcutaneous tissues of man appear to be of the same order as those found for the other homothermal animals, namely, a CO_2 tension between 40 and 55 mm. and an O_2 tension between 15 and 30 mm.

SUMMARY

The tensions of O_2 and CO_2 in the subcutaneous tissues of five normal subjects, studied by the gas depot method, were of the same order as those found by Campbell for other homothermal animals, namely, CO_2, 41 to 50 mm. Hg; O_2, 15 to 27 mm. Hg.

Final equilibrium between the gas pocket and the surrounding tissues did not occur until the foreign body reaction had subsided, usually five to seven days after the initial injection of the gas.

Once equilibrium has been established, the partial pressure of CO_2 remains practically constant, whereas the tissue tension of O_2 shows rather a wide latitude of daily variation.

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THE EXCITATORY PROCESS IN THE MAMMALIAN VENTRICLE

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The pioneer work on the excitatory process in the mammalian heart was performed by Sir Thomas Lewis (1915), and so careful and accurate were his observations that they stand unchallenged to this day. Previous investigators, such as Waller and Reid (1887), Bayliss and Starling (1892), and Gotch (1910) were handicapped by less satisfactory operative technique and by the imperfections of the capillary electrometer, for which Lewis substituted the string galvanometer of Einthoven.

During the years following Lewis' discoveries many additional observations have invited some modification in the interpretation of his data. The necessity of revision is based not so much upon improvements of physiological apparatus and technique as upon the newer observations in the allied field of anatomy. Lewis was led to assume the "radial conduction" of excitation from endocardium to epicardium throughout the ventricle. If, as more recent studies suggest, the anatomical conduction paths lie axially along the direction of muscle bands (extending in the superficial muscles from the apex toward the base of the heart), the timing of surface negativity may bear new significance. The evidence tending in this direction includes anatomical studies, measurements of the spread of excitation along given muscle bands, estimation of conduction rates which are highly consistent, and the experimental alteration of conduction paths.

Todd (1928), Abramson and Cardwell (1931) and Wahlin (1928), among others, believe that there is a communication between the Purkinje material of the two ventricles. To what extent the lack of this concept of the distribution of the special tissue influenced Lewis' interpretation of the excitation process is indicated by the statement, "Since the impulse passes to the ventricle by two distinct channels, the right and left divisions of the bundle, and since no anatomical union is known to exist between the arborizations of the two sides, it might be assumed that the spread to a given ventricle is distinct and confined to that ventricle: that is to say,

the wave does not normally cross the interventricular groove[1] but right and left waves meet somewhere in the septum" (6, p. 95). But this assumption cannot be accepted without question in the light of recent cardiac histology.

As the result of studies concerning the muscular architecture of the heart by MacCallum (1900), Mall (1911), Tandler (1913), and still more recently by Robb (1934), together with the histological studies already mentioned, the thesis may then be offered, that the excitation process may cross the interventricular groove and that conduction may take place in a direction parallel to the fibers of any particular muscle bundle.

To prove this it will be necessary to answer the objections to such a theory as presented by Lewis. His experiments have been repeated with certain additional experimental precautions which will suggest an entirely different interpretation of the data acquired.

EXPERIMENTAL METHOD. Nine experiments each on monkeys (Macacus rhesus) and dogs have given highly consistent results. In all experiments the animals were anesthetized with 50 mgm. per kilogram of Pentobarbital and were then fastened belly up on an animal board. The sternum and the anterior attachments of the ribs were removed to allow sufficient exposure. The pericardium was opened and stitched to the wound, thus making a snug cradle for the heart.

The indirect electrodes were silver coated with silver chloride, and were stitched under the skin. Such electrodes with the standard leads have a resistance of scarcely 200 ohms and are non-polarizable.

The direct electrodes were small glass tubes holding a central woolen wick. The tubes were plugged with a gelatine saline mass. Above the gelatine plug was a saturated solution of copper sulfate into which dipped a copper wire. The wicks extended somewhat beyond the tubes into flexible rubber tubes whose internal diameter was 2 mm. These wicks were attached to the heart by a single very superficial stitch and the rubber tube brought to the heart's surface, thus allowing free movement of the heart, with no possibility of short-circuiting, and at the same time absolutely identifying the point of contact. Such electrodes are non-polarizable, judged by a lack of drifting, and by the fact that upon standardization with either one or ten millivolts, the string is "dead beat." The resistance of these electrodes is low, depending upon the length of the wick, the column of gelatine, etc., but was never above 2000 ohms in these experiments. For the indirect leads a string tension of 1 cm. = 1 mv. was always employed. For the direct leads the string was far tighter, 1 cm. = 20 mv.

Three string-galvanometers, connected by a common ground (Wilson et al., 1934) and mounted on a single table, were procured from the Cambridge Instrument Company. Aside from this ground connection, the

[1] Authors' italics.

three units were entirely separate. By means of prisms the three string shadows are thrown upon a 12 cm. camera. A single rotary time marker interrupts the lights from these units, thus registering simultaneous events in all three records.

Many direct electrodes were applied to the surface of the heart at the beginning of a given experiment so that any desired combination of contacts might be obtained in rapid succession.

Drawings were made of the living heart during the experiment with the position of the electrodes accurately measured and marked in. When the electrodes were removed the contact stitch was left as a permanent marker.

TABLE 1

Times of initial negativity in seconds, relative to R in lead 2

MUSCLE	CONTACT NUMBER	EXPERIMENT NUMBER										
		1	2	3	5	8	9	M 11	M 12	M 13	M 17	M 18
Superficial sinospiral	L. Apex											
	↓ 1	0.005	0.008	0.011	0.004	0.009	0.010	0.002	0.001	0.010	0.010	0.001
	2	0.010	0.010	0.015	—	0.015	0.014	0.006	0.004	—	—	
	3	0.013	0.013	0.030	0.015	0.024	0.027	0.012	0.016	0.024	0.024	0.019
	4	0.015	0.015		0.023							
	5	0.023	0.019									
	6		0.024									
	R. Base											
		4	6	7	M 10	M 14	M 15	M 16				
Superficial bulbospiral	L. Apex											
	1	0.008	0.003	0.005	0.009	0.009	0.007	0.005				
	↓ 2		0.014	0.014	0.019	—	0.019	0.022				
	3	0.025	0.021	0.026		0.024						
	4	0.035	0.029	0.028								
	5	0.043										
	L. Base											

Subsequently each heart was dissected to confirm the position of the contact upon the muscle band for which it was intended, thus avoiding any doubt as to the precise location of each direct contact.

Lewis' criteria for differentiating intrinsic and extrinsic waves (loc. cit. (1), p. 190–191) have been adhered to. All the tracings have been read with the aid of a Cambridge Instrument Company's record measuring instrument. At least three and often six cycles are read from each tracing. Readings were made independently by two people, one of whom had no knowledge of the detail of the experiment. The magnification was such that 0.001 second could be read as 1 mm. on the scale.

RESULTS. *Initial negativity.* There has been no difficulty in dupli-

cating Lewis' data. Our technique differed from his in that for a given experiment all the electrodes were placed along a single muscle, in a line parallel to the fibers of that muscle. In table 1 are presented readings from several experiments. It will be noted that without exception, the times increase along the muscle from the apex toward the base of the heart. Furthermore this sequence holds for points along the superficial sino-spiral muscle which crosses the anterior interventricular groove from

TABLE 2

ANIMAL†	CONTACT	INTERVAL	DISTANCE	CONDUCTION RATE*
		seconds	*mm.*	*mm. per second*
D 1	1 direct electrode at left apex on superficial sino-spiral muscle	0.005	9	1,800
D 2		0.016	45	2,812
D 3	Indifferent electrode on right leg	0.019	54	2,842
D 5		0.019	49	2,579
D 8		0.015	49	3,260
D 9		0.011	20	1,818
M 11	1 direct electrode on superficial sino-spiral near right base	0.010	26	2,600
M 12		0.015	30	2,000
M 13	Indifferent electrode on left arm	0.014	30	2,163
M 17		0.014	26	1,890
M 18		0.018	37	2,060
D 4	1 direct electrode at left apex on superficial bulbo-spiral	0.035	100	2,857
D 6		0.026	71	2,730
D 7		0.023	70	3,435
M 10	1 direct electrode on superficial bulbo-spiral near its origin at the conus	0.009	26	2,600
M 14		0.015	29	1,933
M 15	Indifferent electrodes as above	0.012	20	1,666
M 16		0.017	29	1,706

* Calculated for maximum distances in each heart.

† The "D" series are dogs, the "M" series Macacus rhesus monkeys.

Conduction rate: Median = 2580 mm. per second. S.D. = ±400. Mean 2375 ±128 mm. per second.

left to right, as well as for points upon the superficial bulbo-spiral muscle which were so chosen that they did not cross the groove.

Conduction rates. If one uses the time intervals given in table 1 and calculates conduction rates to the furthest electrode, such values as are shown in table 2 are obtained. If the intervening distances (i.e., between electrodes) are used similar rates are found, table 3. It is stressed that this method of measuring conduction rates is valid only if it can be proven that the points lie along a pathway of the action current. This we have proven by cutting between the contacts. If the intrinsic deflection at the

more basal contact is delayed, or fails to appear, it is presumptive evidence that the action current normally passed from the one area to the other.

TABLE 3

DOG	CONTACT	CONDUCTION RATE
		mm. per second
CII	1–2	2,500
	2–3	2,000
	3–4	2,500
CI	1–2	2,000
	2–4	1,300
	3–6	2,812

TABLE 4

(I.e., table VIII, Lewis, recalculated)

Transmission rates across interventricular groove

DOG	CONTACTS	CONTACTS APART	NATURAL BEAT		EXCITED BEAT
			Interval	Transmission rate	Transmission rate
		mm.	*seconds*		
G. F.	Aortic base to conus	25	0.0241	1,037	432
G. I.	Aortic base to conus	23	0.0018	1,278	607
G. I.	L. V. to mid R. V.	21	0.0082	2,562	894
G. I.	L. V. to apex R. V.	19	0.0099	1,922	795
G. J.	L. V. to central region R. V.	19	0.0097	1,959	671
G. J.	L. V. to central region R. V.	37	0.0091	4,066	480
G. K.	Trabeculated area to mid conus	22	0.0080	2,750	333
G. K.	Mid L. V. to mid R. V.	22	0.0174	1,265	386
G. L.	Trabeculated area to R. V.	23	0.0050	4,600	452 392
G. M.	Trabeculated area to R. V.	22	0.0072	2,930	416
G. M.	L. V. to mid R. V.	24	0.0082	2,925	423
G. N.	L. V. to mid R. V.	17	0.0133	1,277	409
G. N.	L. V. to trabeculated area	14	0.0112	1,250	754
G. O.	L. V. to mid R. V.	17	0.0192	885	627
G. O.	L. V. to trabeculated area	10	0.0118	848	500

Considering the transmission rates for natural beats, and without regard to apparently negative or positive values with reference to R of lead 2, transmission rate in millimeters per second =

Natural beats:	*Excited beats:*
Median 1922	Median 460
Mean 2103 ±230	Mean 523 ±40

Only experiments satisfying these requirements are accepted, when calculating conduction rates.

For comparison with these figures (table 4) we have transcribed table 8 of Lewis and Rothschild (1915) and have calculated the rates for normal beats, which he did not do since he assumed the conduction paths to be radial instead of axial. His "conduction" rates are for excited beats only, and as shown by De Boer (1925) were doubtless slowed by cooling. It is readily seen that his values for *normal* beats are of similar magnitude

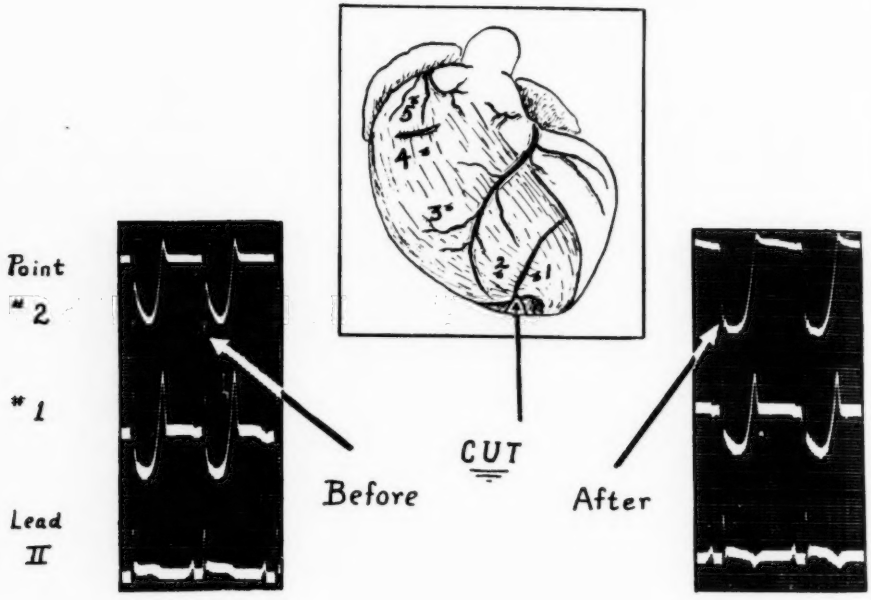

Fig. 1. The sketch shows the superficial sino-spiral muscle on the anterior surface of the heart, crossed by the anterior descending branch of the left coronary artery. The numbers indicate the attachments of the direct electrodes to the surface of this muscle (table 5). The positions of cuts transverse to the muscle fibers are indicated. The electrocardiograms at the left were taken before, while those at the right were taken after, cutting between points *1* and *2*. The intrinsic wave is absent at point *2* after the cut is made. In each instance the upper record is that from point *2*, the middle record from point *1*, and the lower—a standard lead *2*.

to those in table 1. There is more variability in his values, presumably since the points were not limited to a single muscle and were not necessarily in a sequence parallel to fiber direction. That is to say, some of these "rates" do not express actual conduction between two points. Still, there are some points, for instance "aortic base to conus" where both points must have been on one muscle.

One of the chief arguments used by Lewis to disprove the theory of conduction along a muscle band and to prove "radial conduction" was his experiment of cutting the muscle between two contacts. Judging by his figure 14, p. 201, the cut was parallel to the direction of the superficial muscle and could not have been transverse to muscle fibers (see Robb, Easby and Hiss, 1934).

In our experiment CI five contacts were placed along the superficial sino-spiral muscle (see fig. 1). A series of tracings was taken in which contacts 2, 3, 4, 5, were successively paired with the indifferent electrode on the left arm, while there was kept for comparison both contact 1, paired with the indifferent electrode at the right leg, and a standard lead 2. A deep cut was made between points 4 and 5 and later a similar deep cut between 1 and 2. In table 5 the readings are given.

TABLE 5

EXPERIMENT	CONTACT	ARRIVAL OF INTRINSIC DEFLECTION		
CI		Normal	After cutting (see text)	
			seconds	
	2	0.008	0.025	
	3	0.019	0.025	
	4	0.032	0.038	
	5	0.045	∞	No intrinsic deflection (see fig. 1)
				After cutting between contacts 1 and 2
CII	2	0.015	>0.037	Practically absent, certainly not earlier than 0.037

If a muscle band is cut, the appearance of the intrinsic wave is delayed cephalad to that cut, or if the whole cross section is damaged, the intrinsic wave disappears (fig. 2).

It seems reasonably certain that in figure 6 of Wilson et al. (1934) the points 1, 11, 10, 8 and 9, were all along the superficial sino-spiral muscle. Their values for initial negativity are of the same magnitude as those presented in table 1 and also increase in value from apex to base, thus: 0.025, 0.028, 0.046, 0.057, 0.077 respectively.

Discussion. Since the publication of Lewis' data, observations have accumulated which cannot be explained according to his interpretation. Barker, MacLeod and Alexander (1930), working on an exposed human heart, failed to corroborate the early times of initial negativity over the surface of the right ventricle, and disagreed totally with his interpretation of "right" and "left-sided" events. The data here presented are not in

accord with the theory of "radial" penetration of the excitation wave. Let us examine the evidence:

A. It is true that the excitation wave emerges radially through the ventricular wall in three localized areas, namely, where the fibers of the superficial muscles penetrate. *a*. At the left apex, the superficial sino-spiral muscle turns in abruptly (anterior horn) and becomes the anterior papillary muscle. This muscle receives a direct branch of the left ramus of the bundle of His. *b*. In a similar manner the superficial bulbo-spiral muscle forms the posterior horn of the apex and penetrates to become the posterior papillary muscle. The posterior branch of the left ramus of the bundle of His connects directly with this muscle. Presumably the Purkinje fibers follow along the muscle bands. One may suppose, then, that the excitation passes along the branches of the bundle, enters the muscle band and follows it to the epicardial surface. *c*. The deep sino-spiral penetrates at the trabeculated area on the anterior surface of the right ventricle to form the right anterior papillary muscle. This papillary muscle receives directly the right branch of the bundle of His. In these regions the concept of radial penetration is anatomically justified.

B. Another argument against conduction along muscle bands, used by Lewis, was that points relatively near together might have greater variance of time of initial negativity, than other points far separated. The observed fact is true. However, two points can lie near together and still be on different muscles. The times of negativity, then, would be very different, depending upon the distance the excitation had travelled along either muscle. Wherever any muscle penetrates the thickness of the wall (as at the anterior and posterior horns of the left apex, and at the trabeculated area of the right ventricle), the times of initial negativity are early. By dissection these areas can be shown to be in direct relation to the branches of the bundle of His. Hence it is logical to expect early times in all such areas even though they be widely separated on the surface since the action current may have travelled simultaneously by several pathways. The course of the bulbo-spiral in reaching the exterior of heart is considerably longer than that of the sino-spiral. This offers an explanation of the fact noted by Lewis, and confirmed in these experiments, that the time of negativity is not the same at all points on the apex. Lewis' values are 0.0001 and 0.0100 second. The thickness of the ventricular wall at the anterior and posterior horns is fairly uniform and hence cannot serve as the explanation of the differences observed.

C. With so many electrodes attached to the surface of the heart, injury currents must be present. Doubtless such currents exert their influence upon the contour of the complexes recorded. For our purpose it is unnecessary to draw any inferences from the contour of the curves. This discussion requires only the measurement of the upstroke of R in lead 2

and the upstroke of the "intrinsic" waves in the direct leads. If injury current had any effect it would not disturb the relation of R to the intrinsic waves.

D. When a superficial incision is made equidistant from two contacts, after which the apical contact is unaltered, while the intrinsic wave is delayed, or even disappears from the more basal contact, it is impossible to explain the discrepancy on the basis of radial penetration. Obviously the action current never reaches the basal contact. If the action current were penetrating from the endocardium to the epicardium, such an incision should have no effect. If it were a matter of excess of injury current caused by the incision, then, since the incision is equidistant from two electrodes, the injury current would affect both contacts equally. This, however, is never the case. The apical contact continues to have an intrinsic deflection whose time relation to R of lead 2 is unaltered (fig. 1).

There seems, then, adequate reason to believe that the action current does pass *axially* along the ventricular muscle bands, in the two superficial muscles, emerging at the apex, and passing thence in a direction parallel to the muscle fibers, toward the base of the ventricle.

SUMMARY AND CONCLUSIONS

1. Simultaneous direct and indirect leads have been recorded from mammalian hearts on a multigalvanometer set-up in which the three galvanometers were connected through resistance to a central terminal.

2. A standard lead II could therefore be accurately compared with any two points on the surface of the heart without introducing error of time or potential of the direct electrodes.

3. When times of initial negativity are read from tracings obtained by placing the direct electrodes along the axis of given muscle bundles, these times on the superficial sino-spiral and superficial bulbo-spiral muscles are found to increase in an orderly manner from apex to base.

4. Data are cited from literature which could also be interpreted as showing progressive delay in activity along the superficial sino-spiral muscle.

5. Conduction rates calculated from point to point along a muscle strand give a mean value of 2375 $\pm$128 mm. per second.

6. Conduction rates for the dog and monkey (Macacus rhesus) are not statistically distinguishable.

7. If any two direct contacts along a given muscle band are separated by a cut, there occurs a delay in the arrival of the intrinsic wave at the contact furthest from the apex. If the whole cross-section of the muscle is involved, the intrinsic wave disappears from the contact furthest from the apex.

8. Evidence is presented that the wave of excitation does cross the interventricular groove.

9. These observations cannot be explained according to the present theory of conduction of the excitatory process in the ventricle, i.e., the "radial penetration" theory of Lewis.

10. These data indicate that the excitatory process is conducted "axially" in the muscles studied along a pathway parallel to fiber direction.

Grateful thanks are due to the Hendricks Fund of Syracuse University, to the Committee on Scientific Research of the American Medical Society, and to the Ella Sachs Plotz Foundation, each of which bore some part in the support of these investigations.

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A STUDY OF SOME DECURARIZING SUBSTANCES

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A recent publication by one of us (Lindsley, in press) reported the striking changes induced by the dimethylcarbamic ester of m-Oxyphenyltrimethylammonium methylsulphate (Prostigmin Roche, a substance closely related to physostigmine) on the electrograms and the mechanograms of skeletal muscles in patients suffering from myasthenia gravis. It was considered of interest to examine the effects of this substance and of physostigmine on fatigued or curarized muscles, the behavior of which grossly resembles that of the muscles of patients with the disease mentioned.

The antagonism between physostigmine and curare has been long known (Pál, 1900; Rothberger, 1901). The electric responses of the muscle, however, have not, to our knowledge, been analyzed in relation to this antagonism. A further study with modern techniques was deemed desirable. Fatigue and curarization have frequently been interpreted as analogous processes (cf. Lapicque, 1926). A comparison of the action of physostigmine in the two cases would confirm or invalidate this analogy.

With the purpose of answering the preceding questions this study was undertaken. We also expected to obtain some information regarding the nature of curarization if other decurarizing agents than physostigmine were investigated. All these issues are, finally, intimately related to the problem of the transmission of the nerve impulses to skeletal muscle.

METHOD. Cats were used, anesthetized with dial (Ciba, 0.7 cc. intraperitoneally). A cannula was inserted into the trachea for artificial respiration. The sciatic nerve was cut on one or both sides, and buried shielded stimulating electrodes were applied to the peroneal or the popliteal branches. One or both legs were fixed by drills in the tibia.

The contractions of the freed tibialis anticus or gastrocnemius-soleus muscle were recorded on a kymograph by attaching the tendon to the short arm of a writing lever pulling against a strong rubber band. The magnification was 7- to 9-fold. The shortening of the muscle was usually less than 0.5 cm. The initial tension was about 100 grams. Calibrations of the myograph showed that the records obtained were practically lineally proportional to the tensions developed.

The stimuli used were shocks from a Harvard induction coil with 5 volts in the primary circuit. Submaximal or maximal shocks were delivered at a frequency of 2 pairs or less per second, timed by a metronome.

When the muscles were stimulated directly, needles were inserted into their bodies and tendons.

The electric responses of the muscle were led to a 6-stage, transformer coupled amplifier by means of concentric electrodes (Adrian and Bronk, 1929). A loud-speaker or a Du Bois oscillograph permitted the amplified responses to be heard or recorded.

All injections were made into a jugular vein.

RESULTS. I. *Prostigmin and physostigmine.* A study of some pharmacological effects of prostigmin as compared to physostigmine has been made by Aeschlimann and Reinert (1931). The close similarity of effects of the two drugs was noted. Our observations confirm those reported by Aeschlimann and Reinert. The doses of the two drugs used (0.3 to 1 mgm. per kilogram) evoked marked autonomic effects: slowing of the heart, salivation, lacrimation, myosis, secretion of mucus in the trachea, micturition, defecation, often vomiting, and sometimes in the males erection and ejaculation. Generalized skeletal muscular twitches (fibrillation) were also manifest.

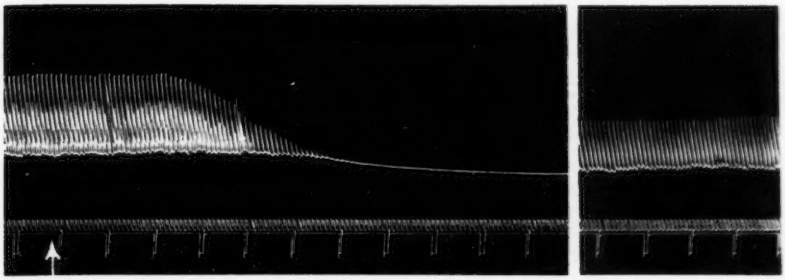
As regards the autonomic effects, the influence on the blood pressure was the only difference detected between the two substances. Physostigmine produced a persistent marked fall of blood pressure, while prostigmin induced only a transient slighter fall succeeded by a recovery to the previous level.

All the autonomic effects of both drugs were abolished by atropine (1 mgm. per kilogram) and uninfluenced by curare.

Because of the satisfactory blood pressure which prostigmin insures, this substance was used rather than physostigmine in the majority of the experiments. The latter drug was tested, however, for all the effects to be reported below. Although the actions of the two drugs were usually similar, certain differences appeared. To avoid unnecessary repetitions, when either of the two substances is mentioned it will be meant to designate both, unless otherwise stated.

II. *Prostigmin and physostigmine on normal or fatigued muscles.* Certain differences between the two drugs were found. We shall first describe the effects of prostigmin.

If prostigmin (0.3 to 1 mgm. per kilogram) was injected while a normal or a fatigued M. tibialis anticus was activated indirectly, a prompt decline to abolition of the responses occurred, succeeded by a recovery to a level usually lower than that prevailing before the injection (fig. 1). Intensifying considerably the shocks applied to the nerve (e.g., from a coil distance of 10, to 4 cm.) did not prevent the depression of the muscular re-



A B

Fig. 1. Adrenals ligated. M. tibialis anticus. Peroneal nerve stimulated at a frequency of 2 pairs of induction shocks per second throughout (coil distance: 11 cm.). In this and the following figures the lowest signal records 5-second intervals.

A, at arrow, prostigmin, 0.5 mgm. per kilogram; B, nine minutes later.

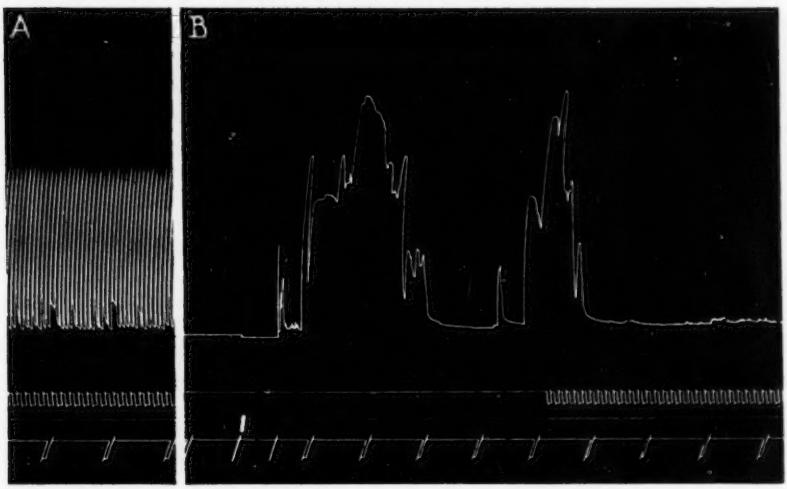


Fig. 2. Atropine, 3 mgm. per kilogram. Adrenals ligated. M. gastrocnemius-soleus. Popliteal nerve stimulated as shown by the upper signal (coil distance 12 cm.).

A, responses before prostigmin; B, at middle signal prostigmin, 0.5 mgm. per kilogram.

sponses. The disappearance of the responses was coincident with the period of generalized fibrillation. The respiration then stopped, probably because the nerve impulses to the respiratory muscles were also ineffective. For this reason artificial respiration was usually started before the injection.

Similar doses of prostigmin, injected while the gastrocnemius-soleus muscle was activated indirectly, elicited likewise a depression of the

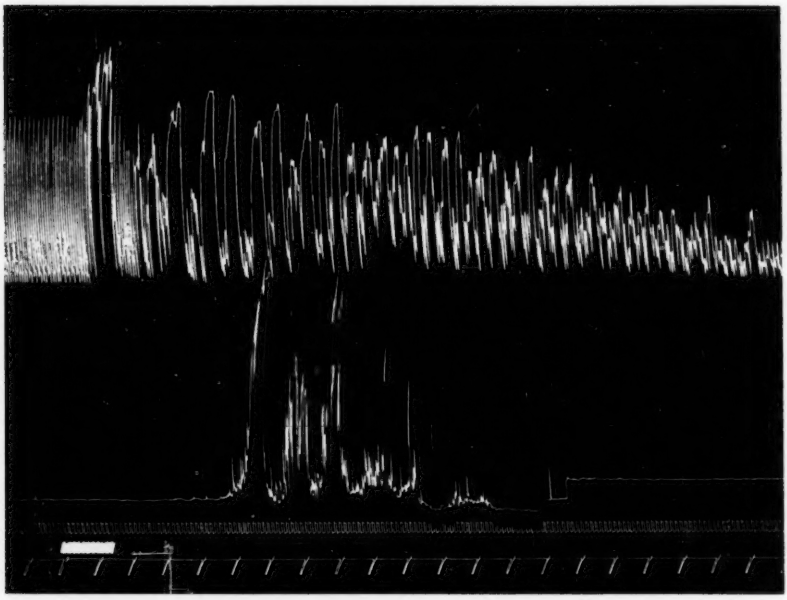


Fig. 3. Adrenals ligated. Upper record left and lower record right gastrocnemius-soleus muscles. Upper signal: stimuli applied to left muscle directly (coil distance 4 cm.). Middle signal: prostigmin, 0.5 mgm. per kilogram.

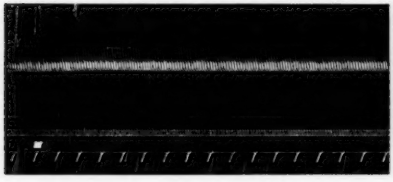


Fig. 4

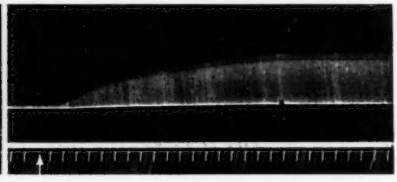


Fig. 5

Fig. 4. Atropine, 1.5 mgm. per kilogram. Curare, 0.5 cc. M. gastrocnemius-soleus stimulated directly (coil distance 4 cm.). At signal prostigmin, 0.5 mgm. per kilogram.

Fig. 5. Curare, 0.1 cc. M. tibialis anticus activated indirectly as denoted by upper signal (coil distance 10 cm.). At arrow prostigmin, 0.3 mgm. per kilogram.

responses. The records were complicated, however, by the occurrence of contractures at the early stages of the decline of the responses. The

refractoriness of the muscle to the nerve impulses outlasted the contractures (fig. 2).

Prostigmin, injected without any background of activation of the muscles, elicited contractures of M. gastrocnemius-soleus (figs. 2 and 3). No unquestionable contractures were recorded from M. tibialis anticus.

Injections of prostigmin while M. tibialis anticus was stimulated *directly* led to a decline of the responses, but never to their disappearance. The original responses were probably due in part to nerve activation, for curare promptly reduced them. On the gastrocnemius-soleus muscle the effects were similar, complicated again, however, by the contractures that developed (fig. 3).

After large doses of curare prostigmin had no effects on the responses of the muscles to direct stimulation (fig. 4).

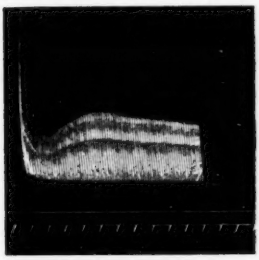


Fig. 6

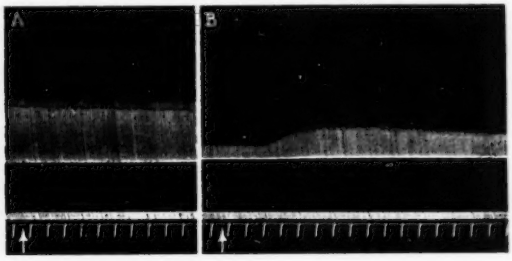


Fig. 7

Fig. 6. Atropine, 1.5 mgm. per kilogram. Physostigmine, 1.5 mgm. per kilogram. M. gastrocnemius-soleus activated indirectly (coil distance 10 cm.).

Fig. 7. M. tibialis anticus activated indirectly (coil distance 11 cm.).

A, before curare and prostigmin. At arrow adrenalin, 1 cc. 10^{-5}; B, after curare, 0.3 cc. and prostigmin, 1 mgm. At arrow same dose of adrenalin.

All the effects of prostigmin described were also obtained after atropine (1 to 3 mgm. per kilogram).

The effects of physostigmine differed from those of prostigmin as follows. No contractures of either muscle were found with physostigmine (0.5 to 2 mgm. per kilogram). Physostigmine had only very slight irregular action on the responses of both muscles to indirect activation.

III. *Prostigmin or physostigmine on curarized muscles.* An injection of 0.1 cc. of the solution of curare employed nearly or completely abolished for about 30 minutes the electrical and mechanical responses of the muscles to nerve stimulation in all the cats tested (cf. fig. 14).

If prostigmin (0.3 to 1 mgm. per kilogram) was given immediately after the paralysis from curare had occurred, a recovery of the responses started usually within less than 1 minute and reached a steady level in

1 to 5 minutes (fig. 5). The responses were then as a rule lower than those before curare.

The antagonism between curare and prostigmin was partly reversible. Thus, after recovery of the responses had been obtained as described above, a new dose of curare again produced paralysis and a subsequent injection of prostigmin once more produced a recovery. On the other hand, if prostigmin was injected before the curare, the same dose of the latter (0.1 cc.) was still capable of causing a similar, although more delayed, paralysis (cf. Rothberger, *loc. cit.*).

In the prostigminized muscles, both before or after curare, the responses to a series of stimuli applied to the nerve after a period of rest varied characteristically. An initial prompt decline was rapidly followed by a gradual and persistent increase (fig. 6).

IV. *Atropine.* This drug was employed to prevent or abolish the autonomic effects of prostigmin. It was also used to eliminate the depressor effects of acetylcholine (see section VI).

Atropine (1 to 2 mgm. per kilogram) did not modify significantly the mechanogram or the electrogram of the normal or fatigued muscles, or produced only minor decreases of both.

If atropine was injected into the curarized animal before prostigmin, the latter no longer induced the prompt recovery described above (section III). Recovery was indeed sometimes so delayed that it was difficult to decide whether it was any quicker than would have occurred without the prostigmin.

When injected *after* decurarization through prostigmin, however, atropine produced only a slight fall of both mechanogram and electrogram, but never a complete paralysis (cf. Rothberger, *loc. cit.*).

V. *Adrenine.* The defatiguing action of adrenine is well known (see Cannon, 1929, for references). With the experimental conditions adopted in the present study and with the doses of adrenine used (1 cc. 10^{-5} or less), this action was slight, sometimes negligible (fig. 7A). Marked transitory increases of both the electric and the mechanical responses to indirect activation occurred, however, from adrenine in the partially curarized or the curarized-prostigminized muscles (fig. 7B).

The responses of the fully curarized muscles stimulated directly were, like those of the fatigued muscles, only very slightly influenced by adrenine (fig. 8).

Cannon (*loc. cit.*) has summarized the evidence which allows the conclusion that the defatiguing effects of adrenine are not exclusively due to blood-pressure changes, but also to a specific effect. Similar controls were made to eliminate the influence of the blood-pressure changes on the decurarizing action of adrenine. Thus, with equal rises of blood pressure adrenine is markedly more effective in the curarized than in the fa-

tigued muscle. When, on injecting adrenine, the blood pressure was prevented from rising by compressing the chest of the cats, the increased responses of the curarized muscles still occurred.

VI. *Acetylcholine.* Atropine was used to avoid the falls of blood pressure which acetylcholine evokes. In the normal or fatigued muscles acetylcholine (0.1 to 1 cc. 10^{-3}) induced a minor, almost negligible transient depression of the responses to indirect stimulation (fig. 9A). After prostigmin this depression was greatly augmented, there occurring usually

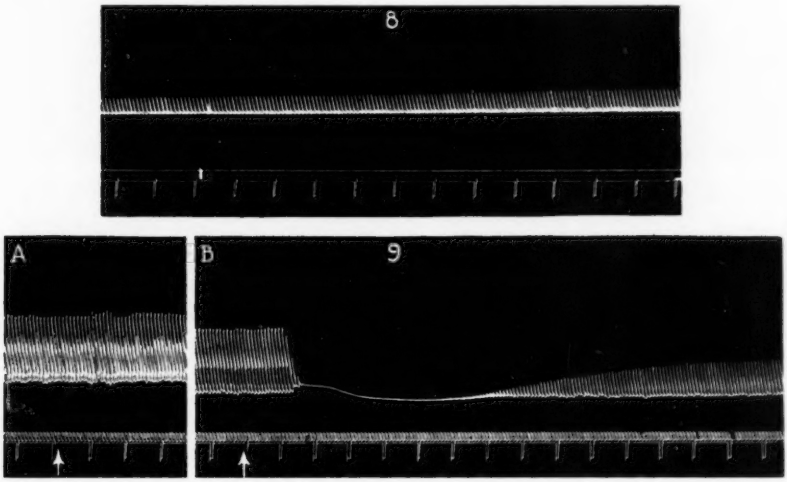


Fig. 8. Atropine, 1 mgm. per kilogram. Curare, 0.6 cc. Physostigmine, 1 mgm. per kilogram. M. tibialis anticus stimulated directly (coil distance 4 cm.). At signal adrenalin, 1 cc. 10^{-5}.

Fig. 9. Adrenals ligated. Atropine, 1 mgm. per kilogram. M. tibialis anticus activated indirectly (coil distance 11 cm.).

A, before prostigmin. At arrow acetylcholine, 1 cc. 10^{-3}; B, after prostigmin, 0.5 mgm. per kgm. At arrow acetylcholine, 0.5 cc. 10^{-3}.

a complete paralysis (fig. 9B). As in the case of prostigmin (section II), intensifying the stimuli applied to the nerve did not prevent this paralysis. There was sometimes a contracture of the muscle coincident with the beginning of the depression of the responses (fig. 12A).

If a small dose of curare (about 0.05 cc.) was added to the preparation just described, acetylcholine elicited a rise, then a fall, and finally again an increase of the muscular responses (cf. fig. 10A). The relative importance of the augmentation or depression depended on the relative amounts of curare or prostigmin injected. If the proportion of curare was greater

the rise was more prominent, the fall being sometimes entirely absent (fig. 10B). With less curare the fall was more significant (fig. 10A).

After atropine and a small dose of curare (and without prostigmin), acetylcholine had only augmenting effects on the muscular responses to

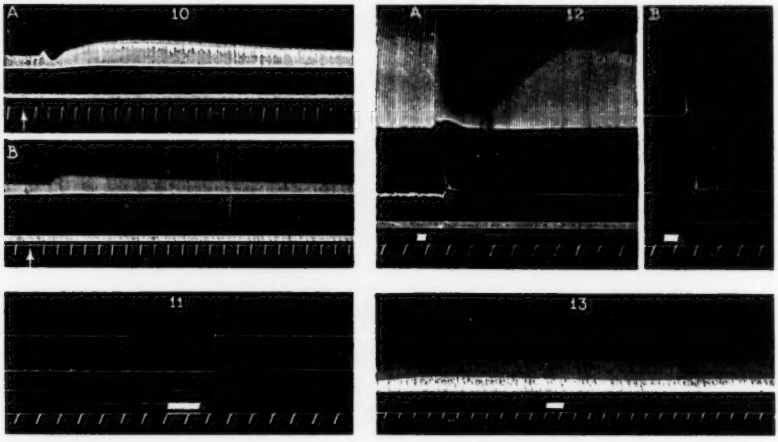


Fig. 10A. Atropine, 1 mgm. per kilogram. Prostigmin, 1 mgm. per kilogram. Curare, 0.1 cc. M. tibialis anticus activated indirectly (coil distance 10 cm.). At arrow acetylcholine, 0.5 cc. 10^{-3}.

B. Adrenals ligated. Curare, 0.3 cc. Prostigmin, 0.5 mgm. per kilogram. Atropine, 1 mgm. per kilogram. M. tibialis anticus activated indirectly. At arrow acetylcholine, 1 cc. 10^{-3}.

Fig. 11. Adrenals ligated. Atropine, 1 mgm. per kilogram. Prostigmin, 0.5 mgm. per kgm. Upper record: M. tibialis anticus. Lower record: M. gastrocnemius-soleus. At signal acetylcholine, 1 cc. 10^{-3}.

Fig. 12. Adrenals ligated. Atropine, 4 mgm. per kilogram. Physostigmine, 1.8 mgm. per kilogram. Upper record: M. tibialis anticus. Lower record: M. gastrocnemius-soleus.

A, upper signal: indirect activation of M. tibialis anticus. At middle signal acetylcholine, 0.2 cc. 10^{-3}; B, at signal acetylcholine, 1 cc. 10^{-3}.

Fig. 13. Adrenals ligated. Atropine, 2 mgm. per kilogram. Prostigmin, 0.5 mgm. per kilogram. Curare, 0.5 cc. Left M. gastrocnemius-soleus stimulated directly (coil distance 0 cm.). At signal acetylcholine, 1 cc. 10^{-3}.

indirect stimulation. The addition of prostigmin again insured the typical picture of rise → fall → rise just described (fig. 10A). Prostigmin appears, therefore, to potentiate the depressing, rather than the augmenting action of acetylcholine.

Acetylcholine, injected after atropine and *prostigmin*, invariably in-

duced a slight contracture of the resting (fig. 11) or activated M. gastrocnemius-soleus. No unquestionable contractures were recorded from M. tibialis anticus in these conditions (fig. 11). When *physostigmine* was used, however, acetylcholine produced contractures of both muscles (fig. 12A and B). These contractures were abolished by curare (fig. 13), but only by doses larger than those necessary for complete elimination of the responses to nerve impulses. The contractures to acetylcholine were also antagonized by adrenine, but again large doses (0.2 to 0.5 mgm.) were necessary.

The action of acetylcholine on the responses of normal or fatigued muscles to direct stimulation after atropine and prostigmin was a partial depression, never a complete paralysis. That the responses, even to strong stimuli, were partly due to nerve stimulation was shown by their decrease after curarization. The height of the twitches after curare was the same as that during the maximum depression by acetylcholine before curare. Acetylcholine after large doses of curare had no effects (fig. 13). We may conclude that acetylcholine has no appreciable influence on the responses of the muscles to direct stimulation.

Tests similar to those described above for adrenine (section V) revealed that the actions of acetylcholine are direct, and not secondary to variations of blood pressure. After atropine, acetylcholine raises the blood pressure (Dale, 1914). These rises are uninfluenced by curare. Yet acetylcholine produced a decrement of the responses to indirect stimulation of normal or fatigued muscles and an increment after curarization (figs. 9 and 10). Again, preventing the blood pressure from rising, by compressing the chest, did not modify the actions described. Finally, when the dose of atropine was not sufficient to abolish all the depressor action of acetylcholine after prostigmin, so that only slight changes of blood pressure occurred, all the effects described were present.

The effects of acetylcholine are specific and not indirectly due to adrenine liberated by acetylcholine from the adrenal glands (Feldberg, Minz and Tsudzimura, 1933), for they persisted after adrenalectomy. Indeed, the antagonizing action of adrenine on the contractures of M. gastrocnemius-soleus was reported above, so that the presence of the adrenals would, if anything, decrease these contractures. Activation by acetylcholine of the peripheral sympathetic neurones (Dale, 1914) distributing to the recording muscles was excluded, since the sympathetic component of the nerves stimulated was disconnected from the corresponding ganglia by section.

VII. *The relations between the electric and mechanical responses.* In all of the variations of the muscular responses reported, whether the variation was an increment or a decrement, the electrogram and the mechanogram changed similarly, i.e., they increased or decreased together. Figures 14, 15 and 16 are illustrative of these parallel variations.

Discussion. *a. The pharmacodynamical effects.* The results reported in section III confirm Rothberger's (*loc. cit.*) conclusions as regards the antagonism of physostigmine and curare. Rothberger stated that atropine did not influence this antagonism. Our data (section IV) lead to the view that atropine opposes the decurarizing action of physostigmine, especially if it precedes the latter substance.

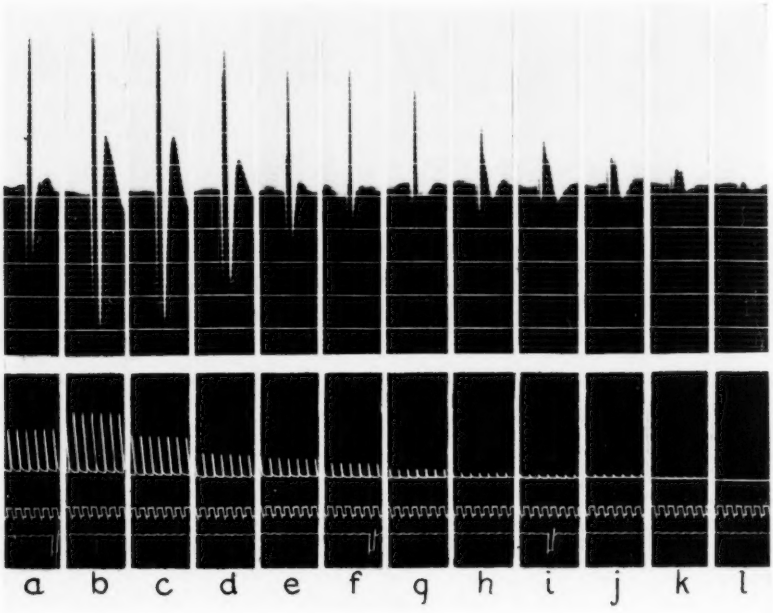


Fig. 14. Effects of curare (0.1 cc.) on the responses of M. tibialis anticus activated indirectly. The stimuli were applied without interruptions. The electric responses correspond to the mechanical records placed below. The injection was made immediately after *a*. The succeeding records correspond to the following intervals in seconds after the injection: *b*, 50; *c*, 95; *d*, 120; *e*, 130; *f*, 145; *g*, 170; *h*, 200; *i*, 240; *j*, 270; *k*, 340; *l*, 415.

The order of injection of the drugs modified their antagonism in the two instances studied: physostigmine-curare (section III) and atropine-physostigmine (section IV). These modifications suggest the possibility that physostigmine acts in these circumstances as it passes through the muscular membrane, perhaps on the membrane itself (cf. Bayliss, 1915, p. 142, for a discussion of other similar phenomena).

Two apparent reversals of action were encountered. Curare changes the depressing effects of prostigmin and acetylcholine on the muscular

responses to indirect stimulation into an augmenting action (sections II, III and VI). It is possible, however, that prostigmin and acetylcholine have two effects—augmentor and depressive—exerted perhaps at different steps in the excitatory process. Curare might then emphasize the augmentor action or abolish the depressive action. If such were the case we would not have here a true "reversal of action" in the strict sense of the expression.

b. Fatigue and curarization. The similarities of the two processes are obvious: progressive decline of both the mechanical and the electric re-

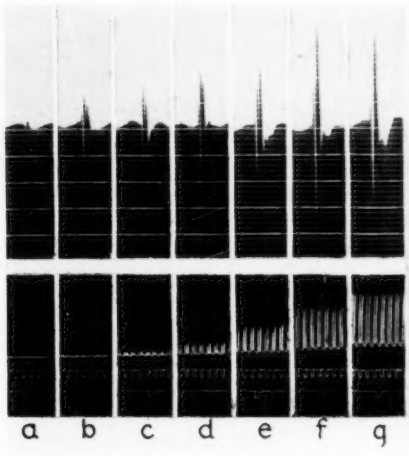


Fig. 15

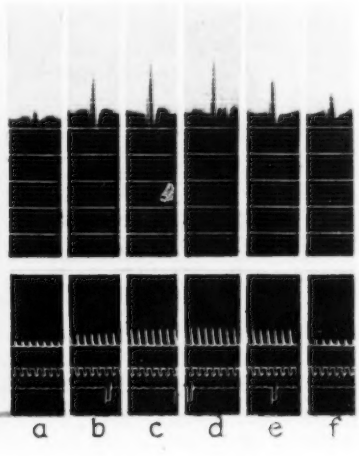


Fig. 16

Fig. 15. Effects of physostigmine (1 mgm. per kgm.) after curare (0.1 cc.). Records as in figure 14. The injection was made immediately after *a*. Intervals in seconds: *b*, 30; *c*, 40; *d*, 60; *e*, 95; *f*, 135; *g*, 205.

Fig. 16. Effects of adrenalin (1 cc. 10^{-4}) after curare and prostigmin. Records as in figures 14 and 15. Intervals in seconds: *b*, 20; *c*, 30; *d*, 60; *e*, 80; *f*, 170.

sponses of the muscles, and final failure of all responses on indirect stimulation while direct excitation is still effective. Lapicque (1926) accounted for these similarities by a uniform explanation. He attributed the impairment and final failure of conduction to heterochronism of the nerve and the muscle, because of a lengthening of the chronaxie of the latter in both cases.

From the present observations, however, we conclude that fatigue and curarization are different processes and that a single explanation can therefore not cover them adequately. The actions of prostigmin (sec-

tions II and III) and of acetylcholine (section VI) bear opposite signs in the two cases. The action of adrenine (section V) is far more striking in the curarized than in the fatigued muscle.

The effects of prostigmin on patients with *myasthenia gravis* (Lindsley, *loc. cit.*) lead to the conclusion that the neuromuscular systems in that condition behave like incompletely curarized, and not like fatigued systems.

c. The mode of action of curare. If Lapicque's theory of curarization mentioned above (*b*) were valid, we would expect that the three decurarizing agents studied (physostigmine, adrenine and acetylcholine) should either shorten the muscular chronaxie, or lengthen the chronaxie of the nerve, or both, thus reëstablishing the isochromism between the nerve and the muscle. The data available do not fulfill this expectation. According to L. and M. Lapicque (1912) physostigmine decreases the chronaxie of muscle before or after injection of curare. Physostigmine should therefore be capable of preventing curarization; but such is not the case (section III, Rothberger, *loc. cit.*). Since prostigmin is a decurarizing agent, it should shorten the muscular chronaxie, as physostigmine. In the fatigued muscle prostigmin should then bring about a recovery according to Lapicque's theory. Experimentally it produces the opposite result (section II). Acetylcholine (cf. Lapicque, 1934) would make the muscle more excitable, and this could explain its decurarizing action (fig. 10). The paralyzing effects of acetylcholine on fatigued muscles before curare (fig. 9B), however, cannot be accounted for by an increased excitability of the muscles. Adrenine at high concentrations (10^{-3}) has been reported to decrease the muscular chronaxie (Lapicque and Nattan-Larrier, 1922); at low concentrations (10^{-5}), however, such as those used in the present observations, adrenine *lengthens* the chronaxie of skeletal muscle (Florkin, 1928). We conclude, therefore, that Lapicque's theory of curarization fails to account for the present data.

Dale and Feldberg (1934) have suggested that acetylcholine is the chemical mediator of the motor nerve impulses to skeletal muscle. If this suggestion is supported by future experiments, and if this chemical mediation occurs at some stage of the excitatory process intermediate between the depolarization of the nerve and that of the muscle—i.e., between the two action potentials—as will be explained below (*c*), then isochromism or heterochronism between nerve and muscle are probably irrelevant as regards conduction of excitation from nerve to muscle. We may assume that the action of curare consists either in impairing the production of the mediating acetylcholine or in preventing the action of acetylcholine on the muscle, and that the decurarizing agents (physostigmine, adrenine and acetylcholine) antagonize or overcome these possible actions of curare. Thus, if curare made the muscle relatively impermeable to the acetylcholine liberated by the nerve impulses at the nerve endings, adrenine

could make it permeable, injected acetylcholine could raise the concentration outside so that sufficient penetration would occur to activate the muscle, and physostigmine could achieve the same effect by preventing its destruction. These assumptions fit the data on hand. They must await, however, further evidence to test their validity.

d. Adrenine. The augmenting effects of adrenine on indirect stimulation of the curarized muscle are markedly greater than those on the fatigued muscles (section V). We may then conclude that the decurarizing and the defatiguing actions of adrenine are independent, a conclusion which is in accord with that reached above (*b*), that fatigue and curarization are different processes.

After curare, adrenine had minor effects on the responses to direct stimulation of the muscle (section V). This lack of marked effects occurred even with submaximal direct stimuli. With such stimuli, a significant increase of the excitability of the muscle would lead to a definite increase of the responses. We conclude that the action of adrenine on the responses of curarized muscles to indirect stimulation occurs at some step in the excitation process prior to the muscle action potential, a conclusion that is in accord with the suggestions made above (*c*) for the mode of action of curare.

e. Acetylcholine. The depression or paralysis by acetylcholine of the muscular responses to indirect stimulation after atropine and physostigmine or prostigmin (fig. 9) is in harmony with the results reported by Wolff and Cattell (1934). These authors observed a slight similar depression in the frog's gastrocnemius perfused with Ringer's solution when small amounts of acetylcholine were added to the perfusing fluid. This depression was independent of a slight contracture of the muscle elicited also by acetylcholine. Wolff and Cattell discuss some possible explanations of this phenomenon and favor the view that the depression is an aspect of the contracture effect.

Relative refractoriness to direct stimulation of different muscles during the contractures developed by acetylcholine has been studied by Gasser and Dale (1926). They found a general correlation between the degree of contracture and the refractoriness to direct electrical activation—i.e., the muscles which develop strong tensions (e.g., the denervated mammalian muscle) become considerably refractory, while muscles which develop low tensions (e.g., the normal fowl) or no contracture at all (e.g., the normal mammalian) are very little or not at all refractory. Gasser (1930), however, has cautioned against the conclusion that refractoriness is proportional to contracture and mentions many exceptions to this rule. In the case we are dealing with here, i.e., indirect stimulation, the discrepancy between contracture and refractoriness is marked. The contracture is small (M. gastrocnemius-soleus) or practically absent after

prostigmin (M. tibialis anticus), while the refractoriness to the nerve impulses may be absolute (figs. 11 and 12).

Acetylcholine does not appreciably alter the responses of the non-curarized, innervated muscles to direct submaximal stimulation (section VI). We conclude, therefore, that its depressant effects on indirect stimulation occur at some stage in the process of excitation by the nerve prior to the stage at which the direct electrical stimulus acts. The two modes of activation are, hence, not directly comparable in judging the mechanism through which acetylcholine exerts its influence.

The opposite effects of acetylcholine, before and after curare, on the responses to indirect stimulation are puzzling. The depressing action before curare is particularly baffling if we accept acetylcholine as the chemical mediator of the motor nerve impulses. The following tentative considerations suggest that the apparent paradox may not be as arduous to overcome as it appears at first sight. If acetylcholine were only effective as a stimulant during its passage through some muscular membrane or when its concentrations at either side of such a membrane should be sufficiently different (cf. Gasser and Dale, *loc. cit.*), then the refractoriness of the muscle to the nerve impulses could be due to lack of passage of acetylcholine or to similar concentrations across the membrane because physostigmine would have made the muscle permeable to the injected acetylcholine (cf. *c*). Curare, by decreasing this permeability (*c*) would reëstablish a difference of concentrations across the hypothetical membrane.

f. The parallel variations of the electric and mechanical responses. Changes of either the action potentials or the contractile tension may be due to changes in the number of active fibers or to variations of these responses in the individual fibers (cf. Davis and Davis, 1932). A knowledge of which of these two modes of variation occurred in the present experiments would give further insight as to the actions exerted by the several substances studied. The data presented (section VII) are not conclusive in this respect. Since the effects recorded appeared only on indirect stimulation of the muscles, obviously it was impossible to test whether some fibers were not effective by using direct stimulation. Only a study of the responses of single muscle fibers may answer the question.

The parallelism of the mechanical and electric responses allows the conclusion that the several depressions and exaltations observed were not due to direct effects of the drugs on the *contractile* mechanism. This conclusion is in accord with the statements previously made (*d* and *e*) that the effects occur at some step prior to the muscle action-potential. The parallelism is also in accord with the suggestion made by Davis and Davis (*loc. cit.*) that the tension developed is determined by the magnitude of the propagated disturbance.

g. The transmission of the nerve impulses to skeletal muscle. Two funda-

mentally different views may be adopted to explain this transmission. The first one, probably prevalent, makes the nerve action-potential the direct stimulus of the muscular action-potential, which in turn directly or indirectly sets off the process that leads to contraction. The methodological advantages of this view, and therefore its appeal, are obvious: conduction in the neuromuscular system would be the same as conduction in the nerve or muscle fiber. Since, however, the nerve impulse may be blocked at the myoneural junction, for instance by fatigue or curare, some hypothesis is necessary to account for this block. Lapicque's (1926) theory of curarization discussed above (*b*, *c*) supplies this hypothesis. Direct tests of the theory have been the subject of recent controversies (see Forbes and Davis, 1935).

According to the second view that may be held as regards the transmission of the motor nerve impulses, there is not a direct continuity of the excitatory processes from nerve to muscle. One, possibly more, intermediate steps occur between the two action potentials. Specifically, the step or one of the steps may be a chemical excitatory agent, possibly acetylcholine (Dale and Feldberg, 1934). The methodological advantage of this view is the greater flexibility of the hypotheses that may be derived therefrom. Furthermore, such a mode of transmission would be analogous with that occurring in autonomic effectors, where chemical mediation has been ascertained (see Cannon, 1933, for references), and in synaptic transmission, where chemical mediation may likewise occur (Kibjakow, 1933; Feldberg and Vartiainen, 1934).

Lapicque's theory has been discussed (*c*) and shown to fail to account for the present evidence. We may add here that we believe that any hypothesis which will include as an assumption a continuity of the neuromuscular excitatory wave of depolarization will similarly fail to cover the present evidence. We base this belief on the conclusions stated previously, that adrenine (*d*) and acetylcholine (*e*) exert some of their actions at some step of the excitatory process prior to the muscular action-potential. Similar considerations apply to the depressing action of prostigmin on the responses to indirect activation (section II). These effects are not due to changes in the excitability of the nerves, for intensifying the stimuli does not modify them (sections II, V, and VI). There should exist then one or more steps intermediate between the nerve and the muscle action-potentials for these actions to occur. The theory of chemical mediation provides an intermediate step and may therefore cover the present data. Further elaboration of this theme must, however, await future evidence.

SUMMARY

The actions of prostigmin, physostigmine, acetylcholine and adrenine on the mechanical and electric responses to indirect and direct stimulation

of normal, fatigued or curarized skeletal muscles were studied in cats. Prostigmin and acetylcholine depress the responses to indirect activation of normal and fatigued muscles (figs. 2 and 9), while they induce an increase of those of curarized muscles (figs. 5 and 10). Under the experimental conditions adopted, adrenine was more effective as a decurarizing than as a defatiguing agent (fig. 7). None of the substances mentioned had any significant effects on the responses to direct stimulation after large doses of curare (figs. 4, 8 and 11).

It is concluded that fatigue and curarization are different processes (p. 63). Lapicque's theory of curarization is shown to fail to account for these data (p. 64). An alternative hypothesis which may account for the data is tentatively suggested. The evidence is discussed in relation to the problem of the transmission of nerve impulses to skeletal muscle (p. 67). It is shown that the data may be compatible with the theory of chemical mediation of these nerve impulses (p. 67).

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HYDREMIA AS A FACTOR IN THE ANEMIA OF PREGNANCY[1]

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The problem of the frequent occurrence of anemia during pregnancy has interested medical practitioners for almost a hundred years. For a review of the literature the reader is referred to the papers of Bland, Goldstein, and First (1) and Strauss and Castle (2). Rowland (3) recently discussed the anemia of pregnancy in its relation to anemia in general. Davies (4) divided it roughly into the hypochromic type with achlorhydria or iron deficiency and the macrocytic type with or without achlorhydria. Ottenberg (5) has also classified it according to type.

While the early investigators were mainly concerned with the therapeutic aspects of the problem, many of them did not neglect to speculate on its probable cause and purpose. Until a few years ago much of the work centered around the question as to whether or not there was an anemia characteristic of pregnancy or whether the anemias observed represented merely a superimposition of the well known anemias upon changes incidental to the pregnant state. Because of the controversial nature of the problem and the lack of a generally accepted standard technic for studying hemoglobin relations, together with the almost exclusive use of the human as a subject, little progress in the fundamentals has been made. However, recent advances in our knowledge of nutrition, particularly with respect to the rôle of the gastric secretions and the part played by Cu has given promise of making future understanding of these anemias more satisfactory. The work of Strauss and Castle (6) and Wills and Mehta (7) has especially led to the expectancy that real progress in the definition of the human types is being made.

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[2] E. R. Squibb and Sons fellow.

Some time ago we had occasion to report on our studies of the anemia of pregnancy in the rat (8). Our work followed that of Mitchell and Miller (9) who investigated the effect of diet upon this anemia. They found it impossible to prevent its incidence by dietary additions of copper, iron, manganese and yeast. We confirmed their findings and in addition failed to get results with supplements of iodine, arsenic, liver, egg yolk, and cod liver oil. In our studies we determined the following: hemoglobin, red cell volume, red cell count, reticulocyte count, water content of the whole blood, refractive index of the plasma, and sedimentation index. On the average we found that on the 20th day of pregnancy there was a 26 per cent drop in the hemoglobin, 20 per cent in the red cell count, and 24 per cent in the cell volume. The water content was markedly increased but not in proportion to the decrease in the aforementioned constituents. In fact, it averaged only a 3.5 per cent increase. This makes it evident that some compensating factors were operative. The reticulocyte count was not definitely increased, nor was the color index changed, but the sedimentation index was very markedly increased as pregnancy advanced. The refractive index increased markedly at first and later dropped to a level below normal.

While it is obvious from our study in the rat that the anemia of pregnancy in this species is at least in part caused by a dilution of the blood, it is understandable why the existence of an hydremic condition was not clearly established long ago. Its manifestations are obviously not uncomplicated. The refractive index for example would not always have revealed it, but by using the simplest procedure, namely, that of direct determination of dry matter in the blood, the hydremic condition could not escape our detection. The aforementioned results have already been discussed at length in a previous publication (8).

In the experiments reported in the present publication we have concerned ourselves with the study of the anemia of pregnancy in the human and the cow. The literature bears frequent reference to dilution of the blood in the human as a cause of the anemia during the pregnant state, but when this work was begun we were unable to find any reference to experimental work which established the existence of the hydremic condition directly. We refer here to direct determination of the water content of the blood. Very recently, however, Oberst and Plass (10) have reported studies on the water distribution in the blood of pregnant and nonpregnant women. They found a higher water content of plasma and red cells in the pregnant state, which was associated with decreased specific gravity and diminished total plasma protein. The cell volume and hemoglobin concentration were decreased, but the hemoglobin concentration in the cells was found to be increased. These results, however, have not as yet been published in detail.

In our present study we have made determinations of essentially the same blood constituents as those determined in our rat experiments. In addition we have also determined the albumin-globulin fraction because we expected first that it would enable us particularly to make an interpretation of the changes in the refractive index and second to obtain an indication of the changes in the water content of the body tissues, since many pregnant women show a palpable edema of the lower extremities and of these only a small number show impaired kidney function.

In our human subjects the blood samples were obtained in the usual manner from the antecubital vein with the least stasis possible. Needless to say, only dry syringes and needles were used. The syringe was emptied partly into a graduated centrifuge tube and partly into a tared weighing bottle. The graduated centrifuge tubes had been coated previously with oxalate by evaporating in them 0.2 cc. of a 2 per cent neutral potassium oxalate solution.

All the determinations were made essentially according to the technic described in our previous paper (8). Water content was obtained by drying the blood to constant weight at 95 to 105°C. Hemoglobin was determined by the Newcomer method (11). Red cell counts were made with a Leitz hemocytometer, using a chamber, cover slip, and pipettes certified by the United States Bureau of Standards. Usually 80 and sometimes 160 small squares were counted. Red cell volume was determined according to the method of Wintrobe (12). Sedimentation index was determined in a Wintrobe tube. Reticulocytes were determined by the method of Osgood and Wilhelm (13). This method was selected in preference to many others because of its accuracy and ease of performance, to which we are able to subscribe after many thousands of determinations. The plasma proteins were determined by the method of Wu (14), as modified by Greenberg (15). We believed this method to be satisfactory because we had no reason to expect a marked variation in the amino acid content of the blood proteins. As a matter of fact, this method has been found quite accurate for plasmas that contained at least 6 grams of total protein. With the exception of two patients, we have found only one occasion where we observed lower values than this. We, furthermore, checked our determinations on numerous occasions by direct Nesslerization after digestion, with good resultant agreement. A comparison of our data with those obtained by others using the same method indicates that our results are in close alignment. We did not make any determinations of the icterus index because data on it have been reported by many others.

Data in a study such as the present can, of course, be collected in two different ways. They can be limited to isolated observations on different patients taken at various times, relying on a sufficient number of observa-

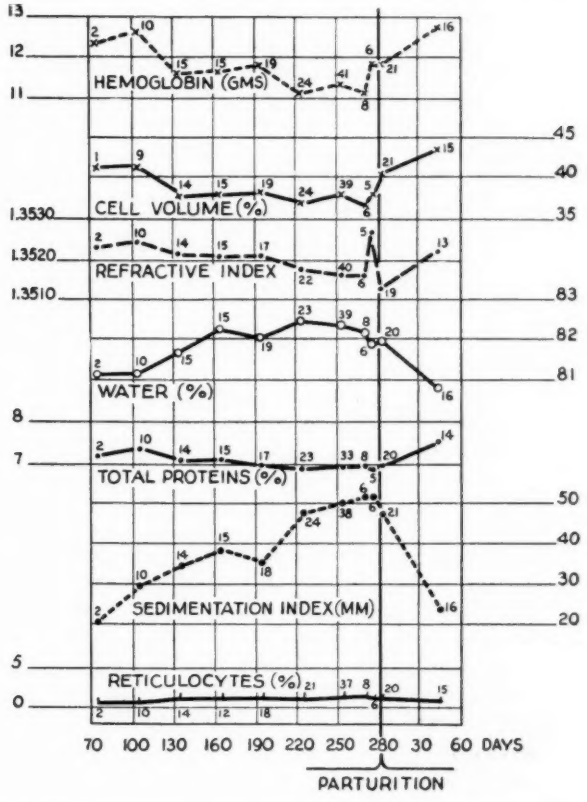
tions to give an average for any period. Data obtained in this manner must be analyzed statistically, accepting certain average results as normal. Another treatment is to obtain data on each subject consecutively for each period of observation. With this technic any variation from a previous status can be taken as an obvious change. In our work, inasmuch as the human patients were continuously available, we followed this second method of study, although we were not able to make the same number of determinations for each period. In many of our patients we continued our observations after parturition to determine subsequent changes. Most of our determinations were made at monthly intervals except during the last month of gestation when we usually made two or three determinations. Although our data are represented as having been obtained on certain days before and after parturition, the exact time was often slightly divergent, yet sufficiently close to the stated time to meet all practical requirements. In all 20 patients were studied. Most of them were from the local Dane County Clinic. Although a considerable number of them were on relief rolls, none of them were under-nourished and none of the pregnancies were complicated by diseased conditions. A small number of patients from the private practice of one of us (E. F. S.) and whose history of nutrition had been ascertained were also included in our series.

Our results on the human are summarized in chart 1. The figures contiguous to the curves represent the number of observations. Of the total average values eight women under continuous observation for 180 days or longer showed a definite progressive anemia. In one case there was a maximum reduction in hemoglobin of 3.5 grams. Two, however, lost only 0.5 and 0.7 gram respectively, which can hardly be called significant, and three were too irregular in their hemoglobin values to allow any deductions as to the actual existence of an anemic state. In the remaining ten patients the period of observation was too short or the determinations were too scattered to allow us to use the values by themselves. Their values were used only in computing the general averages.

The average values reveal in brief that as a definite anemia was produced there occurred a simultaneous increase in water content and a definite fall in cell volume and refractive index. Both the hemoglobin values and the blood solids reached their maximum change at 220 days. The cell volumes, sedimentation index, and refractive index did not differ from this markedly. However, the process of blood dilution was not an uncomplicated phenomenon, because the reduction in protein content was found to be far less than the reduction in total solids. We failed to find any increase in reticulocytes. This harmonizes with our observations on the rat (8). Since we made these observations we have carried out some experiments to determine if the rat is nevertheless capable of producing reticulocytes upon proper stimulation, as by bleeding. We found that

on withdrawing 3 cc. of blood from a 200 gram rat 4 days pregnant there occurred a 9 per cent increase in reticulocytes by the eighth day. A second and a third bleeding accentuated this. At the same time there occurred a pronounced fall in hemoglobin and cell volume. An example

CHART I. PREPARTUM AND POSTPARTUM CHANGES IN THE COMPOSITION OF THE BLOOD OF WOMEN



of these results is shown in detail in table 1. These results show that the hematopoietic centers were well capable of responding to an emergency which demanded an increase in red blood cell formation. The absence of any great increase in reticulocytes in the pregnant state, together with the

rapid restoration of the blood to normal after parturition and the absence of any gross symptoms of anemia, strengthens the probability that the anemia of pregnancy in the rat is not caused by a destruction of red blood cells.

We included the cow in our experiments because we anticipated that the hydremic condition in pregnancy might in general be related to milk production and in that event it was to be expected that in the dairy cow with the enormous volume of milk produced after parturition there might be an earlier accentuated dilution of the blood as compared with the human. The similarity in the length of the period of gestation and lactation and the fetal-maternal weight relation made such a comparison seem especially apropos.

TABLE 1

Reticulocyte response in the pregnant rat to bleeding

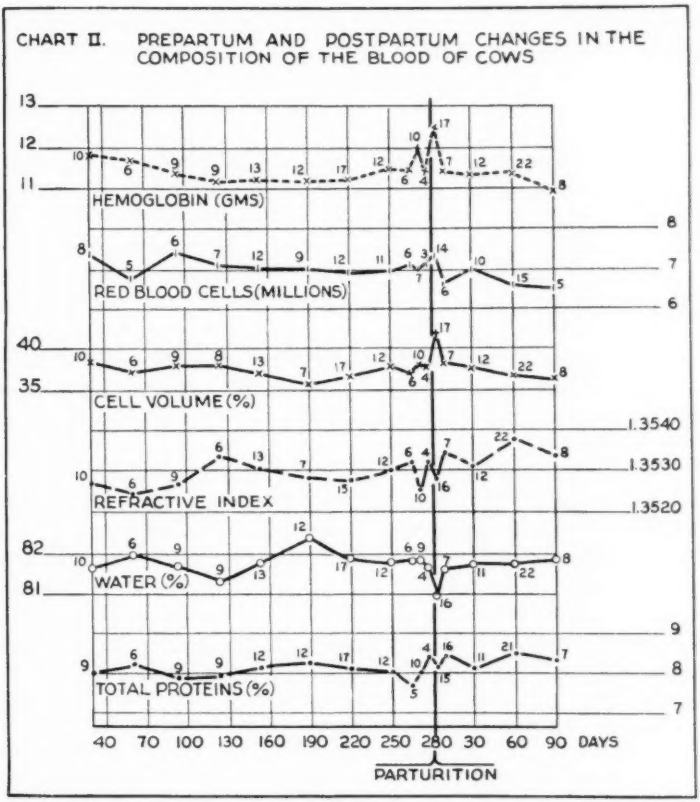
PREGNANT FEMALE NO. 111	WEIGHT	DAY OF PREGNANCY	BLOOD DRAWN	HEMOGLOBIN	CELL VOLUME	RETIC. OF R.B.C.
	grams		*cc.*	*grams*	*per cent*	*per cent*
First bleeding	203	4	3.0	14.32	51.23	0.5
3 days later		7				2.6
Second bleeding	203	8	4.0	10.84	39.53	9.0
3 days later		11				13.5
8 days later		16				14.0
Third bleeding	244	18	4.0	8.30	28.34	13.6
3 days later (parturition) (6 young)		21		5.76		3.6
8 days later				7.96		14.6
22 days later				12.35		

In all we used about 40 cows in our studies. Some of these were followed from the first month after breeding; others were brought under observation later. Blood was obtained with a large stout needle from the jugular vein by turning the cow's head sharply to one side and applying a tourniquet. The blood was allowed to flow freely for a few seconds, after which a sample was collected in a paraffined test tube. From thereon the procedure was the same as with the human samples.

We made some attempts to determine the icterus index, using varying dilutions of potassium dichromate as a standard, but we ran into difficulties with the plasmas of the cows because of their appreciable content of carotenoid pigments. These pigments, of course, obscured the values for bile pigment.

The results of our observations on the cows are shown in chart 2. In contrast with our data on the human, the reduction in hemoglobin with the development of the fetus starting from 40 days after breeding was

very small. It amounted approximately to only 0.6 gram per 100 cc. The red cell count and cell volume showed only a very slight decrease. The total proteins were definitely constant. The water content showed no definite increase and the refractive index possibly increased slightly. To our mind all these trends in our data were not sufficiently pronounced to merit further analysis. Even our evidence for the proof of an anemia



of pregnancy may be considered doubtful in the cow because our determinations, while mostly made consecutively on the same animals, were not always made in this manner nor were the determinations the same in number for each period represented. Data on the sedimentation index were not obtained because the formed elements in cow's blood failed to settle appreciably even after one hour's standing.

The post-partum hematological changes in the cow were also very

minor in character, after the immediate effects of parturition had subsided. It might have been expected that the enormous mammary activity in the cow would have caused a much greater fluid disturbance. The weight of milk produced by our cows ranged on the average from 38 pounds at 30 days after parturition to 14 pounds and 7 pounds at 120 and 30 days respectively before parturition. The remarkable constancy of the different values under observation in the cow indicates that the strain of lactation is met on a much more even physiological plane than is the case with the human. Another marked difference between the cow and the human is the absence of reticulocytes from the blood stream. We made repeated attempts to find these elements in the blood of the cow, but so far have failed to detect them.

It is interesting that the fall in hemoglobin in our human subjects was not absolutely progressive up to the time of parturition. Kühnell (16) also has reported this. He associated this phenomenon with the effects of a late diuresis. While our data do indicate a decrease in water content with advanced pregnancy, the water curve, as a matter of fact, assumed a distinct convexity for many individuals. The increase in hemoglobin was not strictly parallel. Our work accordingly gives no support for Kühnell's suggestion that there is a sufficient regularity in the increase in hemoglobin during the 34th week of pregnancy to permit its being used for determining the period of a given pregnancy.

SUMMARY

Determinations on the blood of normal pregnant women revealed an increase in water content with a concomitant fall in hemoglobin, cell volume, and refractive index. As these data are similar to those previously obtained with the pregnant rat it was considered possible that the hydremic condition might be general in the pregnant state. The dairy cow however proved to be an exception. Apparently in this species physiological adjustments to changes in fluid secretion as well as to pregnancy are more easily made.

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NOTE UPON CROSSED REFLEXES IN THE ACUTELY SPINAL CAT

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The immediate effect of spinal transection upon the crossed reflex of the cat is important in the interpretation of spinal shock. Recently two conflicting views have been expressed upon this subject. McCouch, Snape and Stewart (2) observed initially crossed flexion at a high threshold which fell progressively. After an interval of from 20 minutes to 2 days this was replaced by crossed extension. On the other hand, Forbes, Cattell and Davis (1) regard crossed flexion as an unusual and anomalous reflex and crossed extension in response to a single afferent volley as greater immediately after transection than that obtaining in the decerebrate state.

Since the difference concerns not merely interpretation but results, it is essential to consider the methods employed. McCouch, Snape and Stewart trusted to visual observation of the response without graphic recording in animals intact save for spinal transection. Forbes, Cattell and Davis employed isometric myograms, usually from the vastocrureus muscle group, and made no attempt to record from a flexor muscle.

The preparation of animals for myographic recording has been developed and described in detail by Sherrington (4). In his hands it includes not only rigid fixation of the myograph and of the bones of origin of the muscles recorded, but also the inactivation of all other muscles of the lower extremities. In the experiments of Forbes, Cattell and Davis, the psoas muscles and the saphenous, rectus femoris, and obtureter nerves were cut. On the other hand, the active hip flexors, tensor fasciae femoris and sartorius were left intact, the latter pair in intimate relation to the recorded vastocrureus. The responses they obtained after transection were small enough to be accounted for by an artefact from the contraction of these flexors.

METHOD. To test the point, decerebrate cats with a laminectomy at the 12th thoracic segment were prepared for myographic recording from quadriceps femoris and from semitendinosus after the method of Sherrington with the exception that tensor fasciae femoris and sartorius were left intact as well as those muscles inserting at the great trochanter which could not be reached readily without section of the fascia lata. The femur was fixed by two drills and the pelvis by a pelvic clamp. The contralateral leg was

denervated. Sherrington stimulating electrodes were placed on the crossed sciatic nerve. Crossed reflexes were elicited before and after

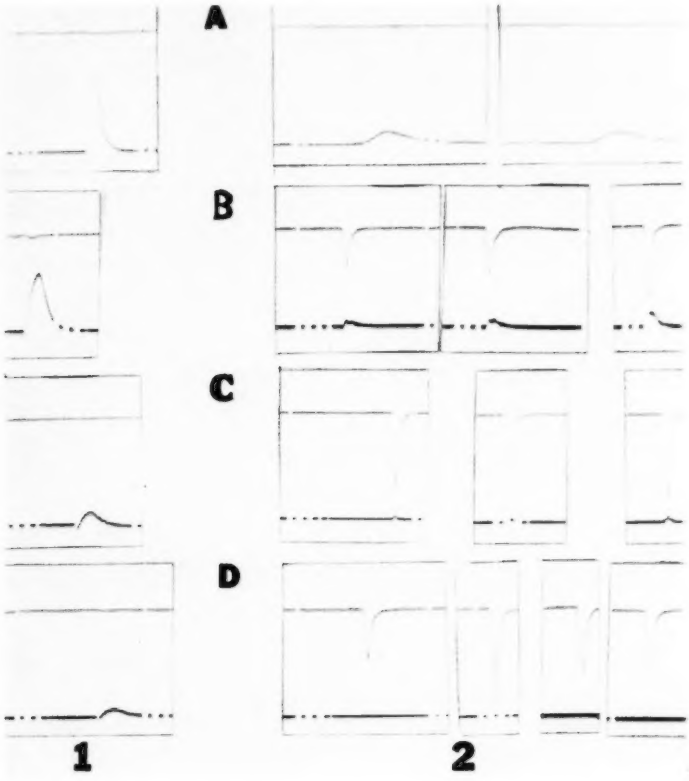

Fig. 1. In all instances the upper line records semitendinosus, the lower line quadriceps. Time in seconds is indicated by cuts in the quadriceps myogram. Quadriceps records upward; semitendinosus downward. Column 1 consists of knee jerks obtained immediately before or after the crossed responses to single induction shocks shown in corresponding lines of column 2. Line A consists of decerebrate responses; line B, of those after cutting the spinal cord under divinyl ether and lowering the table 3 mm. to raise initial tension; line C of those after cutting the nerve to sartorius; and line D of those after completing the dissection. Tension in quadriceps is 71 grams per millimeter, in semitendinosus 49 grams per millimeter as reproduced.

section of the cord. Then the principal nerve to sartorius was cut, and a free resection made of sartorius, tensor fasciae femoris, and the remaining

muscles inserting about the great trochanter. The crossed reflex was again recorded. Five experiments were performed, in the last two of which records were obtained after section of the external branch of the femoral nerve before resection of the fascia lata. Although not mentioned by Forbes, Cattell and Davis the section of this nerve and of the muscles inserting about the great trochanter is customary with these authors. Its section does not, however, ensure complete denervation of sartorius. Knee jerks were recorded at intervals as an index of the reflex condition of the preparation. These were elicited by a mechanical tapper which delivered an upward blow of constant intensity on the lever shank.

Results. All five experiments were concordant in showing crossed extension before and crossed flexion after spinal transection. In four of them, after transection, the small responses similar to those interpreted by Forbes, Cattell and Davis as crossed extension were obtained with the quadriceps lever prior to section of the fascia lata. These were synchronous with larger responses of semitendinosus. When initial tension was excessive, this artefact, although reduced, was not completely eliminated by resection of fascia lata and sartorius. This may result from inadequacy of fixation of the pelvis by a single clamp. On the other hand, at very low initial tension the artefact did not appear. Characteristic records from one experiment are reproduced.

The decerebrate crossed extensor reflex (A2) is not associated with movement of the flexor lever and is far more prolonged than the artefact recorded after cutting the cord. This artefact is double when sartorius is innervated (B2); single and small (like the responses obtained by Forbes, Cattell and Davis) after section of the external branch of the femoral nerve (C2); absent after free resection of fascia lata, sartorius, and the remaining muscles inserting upon the great trochanter (D2).

In spite of deterioration in the reflex state of the preparation as indicated by the diminution in the knee jerk, the threshold for the crossed flexion reflex fell progressively from a resistance of 30 ohms in the primary circuit five minutes after transection to a resistance of 200 ohms at the end of the experiment.

Discussion. Were the interpretation offered by Forbes, Cattell and Davis of their experiments valid, it would necessitate a drastic revision of the classic theory of spinal shock. The importance of this consideration constitutes the sole excuse and even necessity for the present note. They have stated the divergence between their view and that which we have ventured to call classic so clearly and fairly that there is no need to recapitulate it here. The subject has been admirably reviewed by E. G. T. Liddell (3).

The necessity for denervation or section of all muscles of the extremity

save those recorded and the desirability of myograms from antagonistic muscles in work of this character cannot be over emphasized.

In employing the knee jerk as an index of the condition of our cats we do not mean to imply that this reflex is unaffected by spinal shock. Liddell (3) has demonstrated the degree to which it is so affected. Yet its susceptibility to spinal shock is so much less than that of the crossed flexion reflex that it is a sufficiently valid indicator for our purpose. Recovery from an initial shock to the crossed flexion reflex is indicated by the sixfold fall in threshold here reported. Crossed extension is temporarily completely submerged by spinal shock and by inhibition from the less depressed antagonistic reflex of crossed flexion (2).

SUMMARY

Myographic evidence is offered for the view that in the cat immediately after transection the contralateral nociceptive reflex is flexion and that this reflex is depressed by spinal shock, though to a less degree than crossed extension.

Evidence recently advanced in support of the opposite view is analyzed and concluded to depend upon an artefact and hence to be invalid.

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THE EFFECT OF BILE SALTS ON THE OXYGEN CONSUMPTION OF DOG TISSUES[1]

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On the basis of present information, the bile salts present the anomalous picture of being both toxic and essential to the organism. The toxic properties are shown by the effects of intravenous and subcutaneous injection while their function in intestinal absorption and the short life of bile deprived dogs argues for their essential nature. The explanation of such a discrepancy may be that the bile salts are not normally present in the general circulation and that their sphere of action on vital organs is limited to the liver and possibly the kidney. It is also noteworthy that the bile salts appear in the bile invariably in the conjugated form—the type of conjugation, with glycine or with taurine, depending upon the species (Schmidt and Dart, 1920). What advantage accrues to the organism as a result of elaborating conjugated rather than unconjugated bile salts? Is the selectivity in conjugation physiologically significant or merely a matter referable to diet?

As an approach to these questions the effect of bile salts, both conjugated and unconjugated, on the respiration of tissue slices of dog liver, kidney, and spleen was determined. The selection of liver and kidney tissue is self-evident. Spleen tissue was included as a control. The choice of species is limited to the dog since the composition of the bile of other laboratory animals has not been well studied. In dog bile the alkali salts of taurocholic acid, probably with small amounts of taurodesoxycholic acid, are found with no evidence of the normal occurrence of the glyco- or unconjugated compounds (Hammarsten, 1904; Jenke, 1931; Schindel, 1932). The advantage of conjugation was studied by comparing the effect of sodium cholate with that of sodium taurocholate; the effect of type of conjugation by comparing sodium taurocholate with sodium glycocholate. The study was extended further to compare desoxycholic acid with cholic acid.

[1] Presented before the 47th Annual Meeting of the American Physiological Society, Detroit, April, 1935.

METHOD. The Warburg apparatus[2] was used for the study of the oxygen uptake of slices of kidney, liver, and spleen. Control values using the usual Ringer-phosphate-glucose medium at pH 7.4 were compared with the values obtained simultaneously upon slices from the same organ in Ringer-phosphate-glucose solution to which the bile salt under investigation had been added. The determinations were made at 37.5°C., in triplicate with readings taken every half hour for a period of three hours.

The measurements were made in air rather than oxygen for an earlier investigation (Marsh, 1934) has shown that over a three hour period respiration is more uniform in air. Dixon (1934), among others, has pointed out that where air is used for rat tissue, asphyxia of the inner portions of the tissue may occur unless extremely thin and fragile slices

TABLE 1

Average $-Q_{O_2}$ values of dog tissue in air

(Ringer-phosphate-glucose medium at 37.5°C.)

TISSUE	AGE OF TISSUE	
	1-10 hrs.	11-24 hrs.
Kidney		**17.9** (15.4-20.4)
Liver	**4.2** (3.7-4.8)	**4.9*** (3.7-5.7)
Spleen	**5.1** (3.6-6.5)	**4.7** (3.9-5.7)

* Based on values from four different livers. The other values are the average of 9 to 12 determinations. The figures in parentheses give the range.

are employed. With dog tissue, however, the lower rate of respiration permits the use of slices of the usual thicknesses for the various organs.

Tissue and tissue Q_{O_2} values.[3] The tissues were obtained from healthy dogs which, for the most part, had been subjected to minor leg operations in the course of surgical studies of joint regeneration. The majority of the dogs were killed by injecting ether into the heart. The tissues were removed immediately after death and preserved in ice-cold Ringer's solution.

Although Q_{O_2} values are available on many tissues, there are relatively few data on dog tissues. In table 1 the average Q_{O_2} values for dog kidney, liver, and spleen, determined in the course of the investigation, are recorded.[4] These values are from a series of 15 dogs and are representative of the respiration of normal healthy dog tissue measured in air.

[2] For description and use of the apparatus, see Dixon (1934).

[3] Q_{O_2} is defined as the change in cubic millimeters of oxygen per hour per milligram of dry tissue. Since oxygen is absorbed, Q_{O_2} is a negative quantity.

[4] The values found compare well with those reported by Krebs (1933) for dog tissue in contact with oxygen-carbon dioxide: $-Q_{O_2}$ (kidney) = 18, $-Q_{O_2}$ (liver) = 6.

Some tissue was obtained from dogs which had been under amytal anesthesia for twelve hours just previous to death, and from one dog which had been killed with illuminating gas. The tissues of all of these dogs showed abnormal results both as to the Q_{O_2} values of the tissues and as to the effect of the bile acids. Owing to the lack of sufficient data, however, no interpretation can be given to these abnormal results. In passing, it should be noted that the abnormalities were most pronounced in liver and spleen tissue.

SOLUTIONS. The concentrations of the solutions of the bile salts in Ringer-phosphate-glucose medium are expressed in terms of millimoles (mM.) to permit ready comparison among the various salts. Solutions of sodium cholate and sodium desoxycholate were prepared by adding the calculated amount of 0.1 N NaOH to weighed samples of the respective bile acids and diluting with the modified Ringer's solution. The slight dilution of the Ringer's solution was considered to have no appreciable effect on the oxygen consumption.

The *cholic acid* used was part of a sample obtained from Dr. J. G. Reinhold and had the characteristics—m.p. 196.6°C. (cor.) and $[\alpha]_D^{20} = 36.9°$—reported by him (Reinhold and Wilson, 1932).

The *desoxycholic acid* was obtained from Riedel-De Haen. After drying to constant weight in a vacuum oven the acid had a melting point of 173 to 176°C. and titrated as pure desoxycholic acid but gave a slight color reaction in the Pettenkofer test. It was used without further purification although it doubtless contained a trace of cholic acid.

Sodium glycocholate. The pure sodium glycocholate of Riedel-De Haen was used. Kjeldahl nitrogen determinations showed a content of 2.21 per cent against a theoretical of 2.37 per cent. A colorimetric comparison against cholic acid according to the method of Reinhold and Wilson (1932) showed the theoretical content of cholic acid.

Sodium taurocholate. The sodium taurocholate was obtained from Hoffman-La Roche. The salt showed a nitrogen content of 2.18 per cent compared to a theoretical of 2.60 per cent. By recrystallizing from absolute alcohol according to the method of Hammarsten (1904, 1925) and drying to constant weight in a vacuum oven, the nitrogen content was raised to 2.45 per cent. Colorimetric comparison of the purified salt with cholic acid showed equivalence.

On standing for several days the solutions of the bile salts developed a turbidity. When these old solutions were used the values for the respiration were greatly different from those obtained with fresh solutions. For this reason only fresh solutions were employed.

RESULTS. The oxygen uptake compared with that of the controls is expressed as a percentage, positive values indicating increased oxygen uptake. The data obtained with spleen tissue in varying concentrations

of sodium glycocholate or sodium taurocholate are typical of the results and are shown in table 2. Each column of this table is the average of two or more determinations with fresh solutions and different tissue.

In the case of sodium glycocholate (table 2A) at concentrations below 2 mM., there was no significant increase in the rate of oxygen consumption. At a concentration of 2 mM., however, the oxygen uptake was at first 11 per cent higher than that of the control but within an hour and a half there was an inversion to a decreased rate. At the end of the three hour

TABLE 2

Effect of sodium bile salts on oxygen consumption of spleen tissue

(Average percentage variation from control values)

A. Sodium glycocholate

TIME	CONCENTRATION IN MILLIMOLES				
	0.2	1.0	2.0	4.0	8.0
hours					
0.5	5	8	11	−5	−17
1.0	7	5	8	−28	−47
1.5	2	3	3	−58	−72
2.0	3	−5	−5	−76	−88
2.5	−4	0	−7	−81	−88
3.0	−1	3	−15	−88	−93

B. Sodium taurocholate

TIME	CONCENTRATION IN MILLIMOLES					
	0.2	1.0	2.0	4.0	5.0	8.0
hours						
0.5	6	6	5	−6	−18	−9
1.0	8	3	−1	−12	−23	−17
1.5	3	2	3	−8	−17	−16
2.0	13	4	−3	−9	−22	−22
2.5	9	0	0	−7	−18	−25
3.0	3	12	1	−9		−27

period the rate had fallen to 15 per cent below the control. This phenomenon—an initial increased rate of oxygen consumption followed by a marked decrease, the increase and decrease being of about the same order of magnitude—was taken to indicate a "critical inhibitory concentration" dividing the stimulatory concentrations from the depressing concentrations.

Concentrations of sodium glycocholate higher than 2 mM. on spleen tissue produced a decreased rate of oxygen consumption from the very beginning. Characteristic of these higher concentrations is a sharp de-

crease in the rate of oxygen consumption until a fairly constant value is reached. With the higher concentrations marked disintegration of the tissue occurs. Because of this loss of weight on the part of the tissue the oxygen absorption values determined for these concentrations are somewhat higher than they should be.

Sodium taurocholate acting on spleen tissue (table 2B) gave a somewhat different set of data. With increasing concentration the relative rate of respiration was unchanged until a concentration of 4 mM. was reached where a definite decrease in the relative rate was observed. Concentrations higher than this produced a very marked depression. The "critical inhibitory concentration" probably lies just below 4 mM. for this salt and tissue.

From similar data for each of the other sodium bile salts acting on the several tissues, "critical inhibitory concentrations" have been selected and recorded in table 3.

TABLE 3

Critical inhibitory concentrations of sodium bile salts

(Concentration in millimoles)

BILE SALTS	KIDNEY	LIVER	SPLEEN
Taurocholate	10	8	4
Glycocholate	5	4	2
Cholate	1	0.5	0.3
Desoxycholate	0.3	1	0.3

DISCUSSION OF THE RESULTS. The order of the resistance of dog tissue to the inhibitory effects of the bile salts as measured by respiration is, in decreasing order, kidney, liver, spleen. An exception occurs in the case of sodium desoxycholate which depresses the respiration of kidney tissue at a lower concentration than when acting on liver tissue. Spleen tissue, the control tissue, shows less resistance to the inhibitory effects of the bile salts on its respiration than the other tissues. Although liver tissue is somewhat less resistant than kidney tissue, the concentrations at which inhibition becomes apparent are of the same order of magnitude.

Although sodium desoxycholate shows a slightly greater depressive effect than sodium cholate, the differences are not great enough to be significant. The well-known ability of desoxycholic acid to form coördination compounds, the choleic acids (Wieland and Sorge, 1916; Rheinboldt, 1926, 1929; Sobotka, 1932), may be involved but it is difficult to develop a rational explanation involving this principle.

As the data of table 3 show, conjugation of cholic acid with glycine decreases its inhibitory effect sufficiently so that a 5- to 8-fold greater concentration can be tolerated. Conjugation with taurine produces an

even more marked effect, a 10- to 16-fold greater concentration of sodium taurocholate being necessary to reach the "critical inhibitory concentration." The concentration of sodium taurocholate necessary to produce inhibition of respiration with liver tissue *in vitro*, however, is far below the concentration of this salt in the bile of the dog. Expressed in millimoles the concentration of sodium taurocholate in fistula bile, calculated from the data of Smith, Groth, and Whipple (1928), varies between 20 mM. and 40 mM., with a median value of about 30 mM.

The definite difference between sodium glycocholate, which is foreign to the dog, and sodium taurocholate points to specificity. The data at hand do not permit an adequate consideration of this question nor does the work of Terao (1933), who investigated the effect of sodium cholate, glycocholate, and taurocholate on the respiration of rat tissue, prove helpful since the composition of rat bile is unknown. It should be noted, however, that Terao found sodium taurocholate to be less depressive than sodium glycocholate and that the concentrations at which depression became evident were much lower than those obtained with dog tissue.[5]

The mechanism of the action of the bile salts in depressing the respiration of tissue is obscure. Uraki (1933) using the Thunberg methylene blue technic to study the effects of potassium cholate on minced muscle and liver preparations attributes its toxicity to action on the dehydrogenases and the phosphatases. In view of his results it seems probable that the site of the action is the enzymatic system rather than the substrate. Whatever the mechanism, the depressive action of the bile salts on tissue respiration may be regarded as related to toxicity. Thus the "critical inhibitory concentrations" serve as a measure of the relative toxicity of the bile salts.[6]

The toxic properties of the bile salts have been the subject of numerous investigations but as Horrall (1931), in reviewing the subject, rightly points out, much of the work has been done with impure preparations, particularly of the conjugated acids. In general the bile salts have been studied for toxicity by determining the lethal dose, by noting the effect on the heart and blood pressure, and by comparing the rate of hemolysis of erythrocytes. In addition to these effects, Hatakeyama (1930) has found that cholic and desoxycholic acids depress the metabolic rate of

[5] According to Terao toxic effects are definite at a concentration of 0.25 per cent, being more pronounced with sodium glycocholate than with sodium taurocholate. Expressed as millimoles this is equivalent to a concentration of approximately 5 mM. The oxygen absorption was measured over a period of one hour only.

[6] A toxic substance might be expected to stimulate respiration at low concentrations and only as the concentration is increased show depression. The values obtained with sodium glycocholate on spleen (table 2A) show slight stimulation at lower concentrations. With liver and kidney tissue the stimulation at lower concentrations was well marked, being at times as high as 28 per cent.

fasting and hyperglycemic rabbits. The majority of workers have come to the conclusion that taurocholic is more toxic than glycocholic acid and in some instances (Horrall, 1931) the latter has been found to be more toxic than cholic acid. Much of this work is open to the criticism that frogs and guinea pigs were used as experimental animals and as yet the composition of the bile of these animals is unknown. Emerson (1928), alone, on injection of sodium bile salts into dogs, finds sodium taurocholate to be less toxic than glycocholate.

In spite of the discrepancies in the literature the trend of the results indicates that the conjugated bile salts are less toxic than the unconjugated. That this should be the case is not surprising since the conjugation fits the general pattern of detoxication processes (cf. Ambrose and Sherwin, 1935). Further the very rare occurrence of the unconjugated bile acids must have some significance. In spite of the vast amount of work that has been done with the bile salts apparently only two investigators have ever detected the unpaired bile acids. Jenke (1928) noted the presence of cholic acid in the bile of a bile fistula dog following the ingestion of a large quantity of sodium cholate, and Schönheimer (1932) was able to detect a small amount of cholic acid in the bile of a man with an open bile fistula and a pathological liver.

Although it is uncertain whether the bile acids are formed as such and then conjugated or whether they come into being in the conjugated form, the results of the effects of the two types on the respiration of tissue show very definitely the advantage of conjugation. The differences between the values found with sodium glycocholate and taurocholate point to a specificity in this conjugation.

SUMMARY

Measurement of the oxygen consumption of dog tissues in Ringer-phosphate-glucose at 37.5°C., respiring in air, show the following average constants: $-Q_{O_2}$ (kidney) = 18, $-Q_{O_2}$ (liver) = 4.5, $-Q_{O_2}$ (spleen) = 5.

With increasing concentration of the various sodium bile salts added to Ringer-phosphate-glucose medium, a "critical inhibitory concentration" is reached beyond which further increase in concentration results in marked inhibition in the rate of respiration. With spleen, liver, and kidney tissues these concentrations are under 1 mM. for the unconjugated bile salts, sodium cholate and desoxycholate. Sodium glycocholate exhibits "critical inhibitory concentrations" as follows: spleen—2 mM., liver—4 mM., kidney—5 mM. For sodium taurocholate the concentrations are: spleen—4 mM., liver—8 mM., kidney—10 mM.

The data show that conjugation serves to diminish the inhibiting effect of the bile salts on tissue respiration. The difference between glyco-

cholate, which is foreign to the dog, and taurocholate suggests specificity in this conjugation.

Interpreting the effect on respiration as a measure of toxicity, the conjugated bile salts are far less toxic than the unconjugated. Whether this indicates that conjugation is a detoxication process is problematical.

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CORRELATED STUDIES OF THE PARTITION OF CALCIUM AND INORGANIC PHOSPHORUS IN THE BLOOD SERA OF EQUIDAE

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The fact that calcium circulates in mammalian blood in 2 distinct forms was first stated by Pribram (1) who differentiated between the diffusible and non-diffusible fractions. It was shown by McLean and Hastings (2) that nearly all the diffusible calcium exists in the ionized form, and it appears that the normal organism possesses a regulatory mechanism directed toward the maintenance of this calcium ion at a physiological level. No complete survey has been made of conditions associated with variations in the behavior of total and diffusible (ionic) calcium. Since the diffusible fractions are assumed to be the more physiologically active, it is apparent that determinations of total calcium and inorganic phosphorus may, in themselves, be of little significance. This paper presents a study of calcium and inorganic phosphorus in the blood sera of certain Equidae from the standpoint of their partition between the diffusible and non-diffusible forms. The non-diffusible fraction represents the difference between the total and diffusible values. The relationships existing between the total, diffusible and non-diffusible calcium and inorganic phosphorus were examined by statistical analysis. The horses used represented 3 distinct tribes falling into widely differing weight groups, which gave the opportunity of studying concurrently possible variations due to body size and to racial composition.

EXPERIMENTAL. Bloods were obtained from mature horses ranging in age from 3 to 30 years. Pearson (3) showed that the inorganic phosphorus in the serum of the growing horse declines up to the time of maturity, when a more or less constant level is reached. In the present study no changes were observed in the total serum calcium and inorganic phosphorus over the period from maturity to senility. Three different breeds of horses were used representing three weight groups as follows: Percherons, over 772 kgm.; Arabians, 408 to 479 kgm.; and Shetlands, less than 249 kgm.

The blood was drawn from the jugular vein and the serum from the individual samples separated from the corpuscles by centrifugalization.

The ultrafiltration procedure used was that of Greenberg and Gunther (4). Calcium was analyzed by the method of Kirk and Schmidt (5), modified in that the calcium oxalate precipitate was redissolved in the same tubes in which the precipitation had been carried out. The inorganic phosphorus was determined by the method of Fiske and Subbarow (6).

DISCUSSION. The summarized results of the calcium and inorganic phosphorus analyses are presented in table 1, which gives the mean values ± the standard deviations. Fifty-seven to 60 per cent of the calcium is diffusible, while 90 to 95 per cent of the inorganic phosphorus is diffusible. As shown by the large standard deviation of the non-diffusible inorganic phosphorus, this fraction is subject to wide variations. The variations do not appear, under normal conditions, to be correlated with corresponding changes in other fractions of calcium or inorganic phos-

TABLE 1

Partition of calcium and inorganic phosphorus in equine serum

GROUP	NUMBER OF ANIMALS	CALCIUM PER 100 ML. SERUM			INORGANIC PHOSPHORUS PER 100 ML. SERUM		
		Total	Diffusible	Non-diffusible	Total	Diffusible	Non-diffusible
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Percherons	26	13.01±1.28	7.67±0.69	5.35±0.99	3.29±0.27	3.13±0.26	0.16±0.14
Arabians	17	13.59±0.62	7.77±0.65	5.82±0.88	3.30±0.25	2.96±0.30	0.35±0.21
Shetlands	18	13.11±0.58	7.73±0.70	5.38±0.80	3.96±0.40	3.67±0.33	0.29±0.21

phorus. In nutritional hypophosphoremia, as pointed out by Pearson and Catchpole (7), there is evidence that the colloidal phosphate exists as tertiary calcium phosphate.

The total inorganic phosphorus content of the serum of the Percheron group was 3.29 ± 0.27 mgm. per 100 ml. of serum as compared with 3.96 ± 0.40 mgm. for the Shetland group. Using Fisher's t value (8) as a test for the significance of the difference between means, there is a highly significant difference between both the total and diffusible inorganic phosphorus of the sera of the Percheron and Shetland groups. The significantly higher inorganic phosphorus content of the sera of Shetlands weighing less than 249 kgm. as compared with the Percherons weighing over 772 kgm., and maintained on similar nutritional regimes, is an interesting phenomenon. Whether or not this difference bears a direct relationship to the function of growth, and is generally correlated with smaller body size within a species, is not indicated by these data. Nor are we able to suggest a physico-chemical basis upon which to postulate a higher phosphorus requirement for smaller animals as compared with larger animals of a species. The inorganic phosphorus content of sera varies greatly among mammalian species. A study of the origin of the Percheron breed

shows a derivation from horses of Arabian blood, while the Shetlands were bred for centuries without intermingling of outside blood. Therefore, it is entirely possible that the differences we have observed are racial and bear no direct physiological relationship to body size.

Unlike the phosphorus analyses, the calcium content of the sera revealed no important differences between the heaviest and lightest groups of horses. The work of Cheymol and Quinquaud (9) on the serum calcium of dogs led to an apparently contradictory result. They divided their animals according to weight into 3 groups, of which only the lightest and heaviest need be considered here. The calcium content of 52 dogs weighing less than 10 kgm. each averaged 10.80 mgm. per 100 ml. of serum, while the average for 12 dogs weighing over 20 kgm. each was 11.10 mgm. These workers concluded that the serum calcium was highest in dogs of greatest weight. In the present work with horses not even a tendency was observed for the serum calcium content in the Percherons, weighing over 772 kgm., to exceed the values in the Shetlands, the smallest of the species weighing less than 249 kgm.

Studies have been made of the biometry of total calcium and inorganic phosphorus of the serum. If the total calcium, diffusible calcium, and non-diffusible calcium rise and fall together, and in about the same relation to one another, it should be possible to show a correlation between them. Likewise, definite biometrical relationships may hold for inorganic phosphorus. Further, if the inorganic phosphorus varies inversely with the calcium of the serum, as has frequently been supposed, a statistical analysis of the data should support this theory, and if such were the case this method might indicate what fractions are involved in this relationship. Accordingly, certain correlation coefficients were calculated for the values observed in each of the three groups of horses and also for the summation of the total of 61 determinations. The results of these calculations for the total determinations were as follows:

r for total Ca and total inorganic P	: $+0.08 \pm 0.13$
r for total Ca and diffusible Ca	: $+0.30 \pm 0.12$
r for diffusible Ca and diffusible P	: $+0.13 \pm 0.13$
r for total P and diffusible P	: $+0.87 \pm 0.03$

The correlation coefficients recorded were not materially different from those obtained when calculated separately for each of the three breeds of horses. These data for Equidae agree with the findings of Palmer, et al. (10) that there is no significant relationship between total calcium and inorganic phosphorus in the blood of dairy cattle. Janson (11) working with human sera also failed to find a constant relationship between total calcium and inorganic phosphorus. Furthermore, the fact that we failed to find a significant correlation between diffusible calcium and inorganic phosphorus indicates that these constituents do not vary inversely, as has been claimed by various workers.

Without the use of statistical analysis Janson (11) concluded that there was no constant relationship between the total and diffusible calcium content of the sera. His observations included cases in which the calcium and inorganic phosphorus were not within the normal range. In equine serum the correlation coefficient $+ 0.30 \pm 0.12$ between total and diffusible calcium is significant. In our series all the blood samples were normal with respect to calcium and inorganic phosphorus content. These results, therefore, do not preclude the necessity of determining both the total and diffusible calcium content of the serum in order to obtain an exact picture of the calcium situation in abnormal conditions.

The high correlation coefficient of $+0.87 \pm 0.03$ between the total and diffusible inorganic phosphorus clearly demonstrates the close parallelism existing between them. Therefore, a determination of the total inorganic phosphorus is in itself sufficient to give a close approximation to the true picture of the inorganic phosphorus situation in the blood of Equidae.

SUMMARY

1. The blood sera of 3 distinct breeds of horses, Percherons, Arabians and Shetlands, were investigated for their content of diffusible and non-diffusible calcium and inorganic phosphorus.

2. Fifty-seven to 60 per cent of the calcium and 90 to 95 per cent of the inorganic phosphorus are normally diffusible.

3. The inorganic phosphorus content of the serum of Shetlands was significantly higher than in Percherons or Arabians.

4. There was no significant correlation between total calcium and inorganic phosphorus; these constituents do not vary inversely with each other.

5. There was a highly significant correlation between total and diffusible inorganic phosphorus, and significant correlation between total and diffusible calcium.

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THE CORONARY BLOOD FLOW IN AORTIC STENOSIS, IN AORTIC INSUFFICIENCY AND IN ARTERIO-VENOUS FISTULA

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Current ideas regarding the modifications of coronary flow in such conditions as aortic insufficiency, aortic stenosis and arterio-venous fistula are based almost entirely upon inferences. Thus Lewis and Drury (1) and more recently Laplace (2) have concluded from studies of the general peripheral circulation that coronary flow is probably decreased during aortic insufficiency and arterio-venous aneurysm, while Bourne (3) has reached the same conclusions by a theoretical analysis of the relation of coronary flow to aortic pressure. Parade (4) has inferred that accompanying the increased activity of the myocardium and the reduced aortic pressure in aortic stenosis, there would be a deficit between the demand and the supply of blood to the left ventricle.

Apparently the only recorded experiments are those of Smith, Miller, and Graber (5) who concluded from studies of coronary sinus outflow that the blood flow through the heart is impaired in these conditions by the fall in diastolic pressure. Indeed their more general conclusion is that "while the systolic phase of the blood pressure may to a certain extent influence the coronary flow, it is certainly subordinate to that of diastolic pressure in maintaining the coronary circulation."

A clearer picture of the changes in coronary flow during these circulatory lesions would be of great clinical importance. Since such disturbances are associated with profound changes in heart beat and blood pressure, physiological information may also be obtained concerning the part these latter factors play in modifying coronary flow. Recently Green, Gregg and Wiggers (6) devised a method which utilizes the principles of differential pressure curves for evaluating changes in coronary flow. This method allows a detailed analysis of the respective rôles that phasic changes in blood pressure and concurrent variations in peripheral coronary resistance play in any flow changes discovered. The method is applicable to the heart *in situ*, with blood supply and, to a certain extent, nerve supply intact.

PROCEDURE. Dogs 10 to 15 kgm. in weight were anesthetized with morphine and sodium barbital, artificial respiration was given, the chests were opened in the midline, and the hearts suspended in pericardial cradles.

The coronary flow was estimated by the method noted above (6) except that in several experiments the heart rate was slowed by clamping the sinus node, after which the heart was driven by an electrical pace maker. This device also controlled the electromagnetic clamp used to compress the coronary artery intermittently during the estimations of systolic and diastolic peripheral coronary pressures.

Aortic insufficiency was produced by the trocar cannula of Wiggers (7) inserted through the wall of the ventricle. Aortic stenosis was produced by an instrument resembling a large Morawitz cannula, with the end sealed. This was inserted through the ventricular wall and so placed that when the rubber tube at the end was expanded, any desired degree of stenosis could be produced at the aortic orifice. When properly applied this procedure is preferable to constriction of the aorta since the obstruction is between the heart and the coronary arteries, and thus more closely simulates a pathological lesion. Arterio-venous fistulae were produced by shunting the blood from the innominate artery into the right innominate vein by means of a large paraffined tube. With each of these procedures it was always possible to restore the circulation to normal. Thus, repeated determinations could be made on the same animal, and records taken during the recovery periods could be checked against the original controls. In order to study quickly the compensatory effects that are possible through increasing peripheral resistance, the aorta was compressed to various degrees by a screw clamp placed around the thoracic aorta just above the diaphragm.

As previously discussed (6) the two pressure curves—aortic and peripheral coronary—recorded during each determination of flow must be redrawn to the same ordinate scale. Since this procedure is laborious, a mechanical device called a coördirectograph was designed to make these adjustments. It consists essentially of a pantograph arranged to enlarge only in a direction at right angles to one of the axes of a curve; i.e., in these records at right angles to the abscissal axis—while the pantograph as a whole is carried across the curve parallel to this axis. As shown in the diagram of the device, figure 1, the collars *H*, *I* and *J* slide along rod *G* and carry punch pointers which may serve as reading, writing, or fixed points, as need be. *G* is fastened to the carriage *F* by which it is transported along the fixed rod *E*. The rods *A*, *B*, *C* and *D* are the pantograph rods. The movable joints *L* and *N* can be fixed at any point along *A* and *C*, and *B* and *D* respectively in order to give the proper degree of magnification. A detailed description of this device is published elsewhere (8).

RESULTS. *The effects of aortic insufficiency.* In the course of six

experiments, thirty-four determinations were made of the effects of uncompensated and compensated aortic insufficiency upon the coronary circulation. The three graphs of figure two, reproduced from one of these determinations, show typical results. The upper curve, *A*, of each graph of this figure and of all subsequent similar figures is a tracing of the aortic pressure curve; the middle curve, *C*, a reconstruction of the appropriate peripheral coronary pressure curve, portrays the resistance offered to flow in the coronary vascular bed; while the lower curve, *D*, obtained by subtraction of curve *C* from curve *A* gives the resultant effective pressure, and therefore the probable velocity of coronary flow. The line *M* in this and all subsequent graphs is the true mean blood pressure, determined by measurement of the area under the aortic pressure curve.

The graph, I, of the normal control shows essentially the same characteristics as the normal curves previously published (6). Thus the velocity curve *D* shows two maxima—one during systole and a higher one during diastole—and two minima—one approximately at the end of isometric relaxation and the other approximately at the incisura. The shaded area underneath the curve, representing the flow during systole measured, on the original enlarged graphs, 1780 sq. mm. while that for diastole measured 2820 sq. mm. The partition of flow between systole and diastole $\left(\frac{S}{D}\right)$ can be expressed by the ratio $\frac{1}{1.59}$, the flow per cycle by the sum 4600, and the minute volume index of flow by the product of 4600 and the heart rate 120 which gives 552×10^2.

Graph II shows the changes that occurred shortly after creation of an aortic insufficiency. The aortic systolic pressure has remained approximately constant but the diastolic pressure has declined 24 mm. Hg and the ejection period has lengthened. The coronary resistance curve, *C*, differs from the normal in that: it does not reach as high a level during systole, it falls to a slightly lower level during diastole and it has not reached as great a height at the beginning of ejection. The velocity curve, *D*, in comparison with the normal, declines much more rapidly during late diastole and during the isometric contraction period. This results in a lower isometric minimum, but the subsequent systolic maximum is higher. The diastolic maximum, although following upon a higher incisural minimum, remains below that of the normal. The relatively increased systolic flow, and the actually reduced flow per beat, resulting from these changes in the velocity curve, are made evident by a comparison of the shaded areas of graphs I and II. Thus in graph II the $\frac{S}{D}$ ratio is $\frac{1}{1.06}$ while the area representing volume flow per cycle is 3490 sq. mm. or 76 per cent of the normal. Since heart rate remained constant this also represents a reduction to 76 per cent of the normal flow per minute.

While these are the typical results of uncompensated aortic insufficiency, a few minor variations obtained in different experiments should be noted. Thus a shortening of the interval between the beginning of the rise of the coronary resistance curve and the onset of ejection was frequently observed. In a few instances of very low aortic diastolic pressure the isometric minimum of the velocity curve was depressed to zero and even below, indicating a complete cessation of flow and the possibility of some regurgitation during the interval of isometric contraction and at times even in late diastole. Measurements of one such case showed a conversion of a normal $\frac{S}{D}$ ratio of $\frac{1}{2.05}$ into a ratio of $\frac{1}{0.49}$ and a reduction of the area representing flow per beat to 50 per cent of the normal.

Graph III illustrates the compensation produced by partial compression of the aorta. Systolic and diastolic pressures have both risen restoring the true mean aortic blood pressure, *M*, to normal. The period of ejection has been further prolonged. Even under these conditions the maximum systolic resistance, curve *C*, is below the normal, graph I, although it is greater than in uncompensated aortic insufficiency, graph II. The minimum level of the curve is slightly higher than in the corresponding part of the normal curve. The velocity curve, *D*, presents the same rapid drop during late diastole that is noted in graph II, but the isometric minimum and the succeeding systolic maximum are both greater. The incisural minimum is not as low as in either graph I or II and the diastolic maximum rises above that of graph II and almost equals that of the normal in graph I. As a result the shaded area representing flow during systole is larger than normal, and that representing flow during diastole is almost equal to normal. This results in an $\frac{S}{D}$ ratio of $\frac{1}{0.96}$ and an increase in the area representing total flow per beat to approximately 110 per cent of the normal.

From such an analysis of the curves of figure 2 it may be concluded that the primary result of an uncompensated aortic insufficiency is a decrease in the coronary flow due solely to the loss of pressure head during diastole, but that this decrease is mitigated by the fortunate diminution of resistance to flow occurring during systole. Since this allows an increased flow during each systole, the extent of reduction of the minute flow is not as great as would otherwise be the case. In addition, the minute volume of coronary flow can be increased to, or even above, normal by compensation involving an increase of the peripheral resistance sufficient to elevate the mean blood pressure to a normal level.

The effects of arterio-venous fistula. In the course of two experiments, five determinations were made of the effects of arterio-venous fistula upon the coronary circulation. The results of a typical determination are reproduced in the two graphs of figure 3. Because of the conditions of the

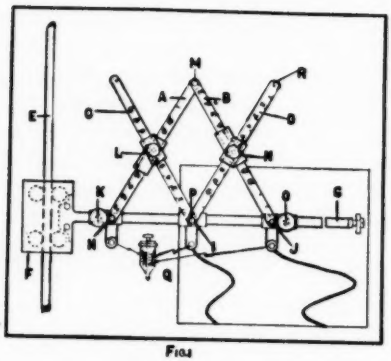

FIG.1

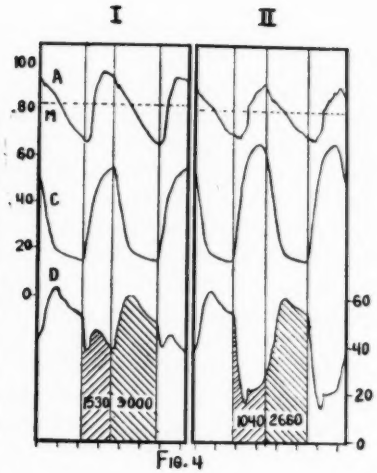

FIG. 4

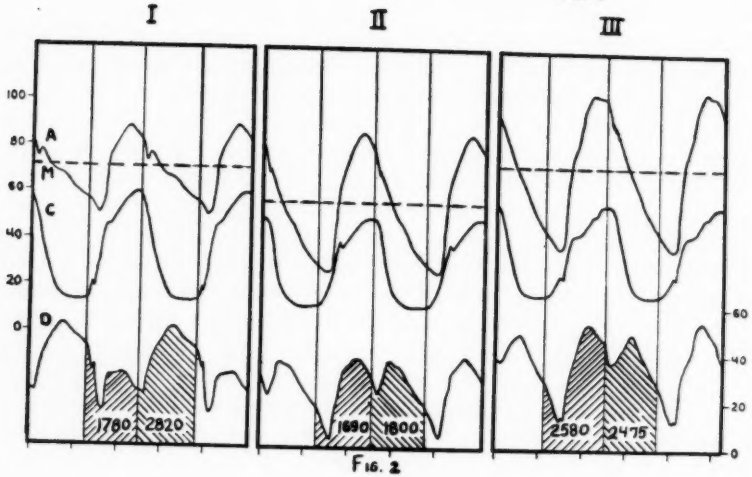

FIG. 2

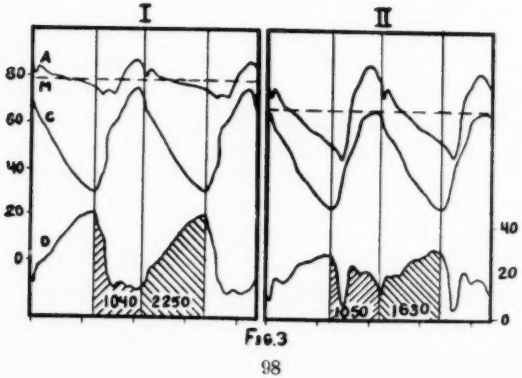

FIG.3

experiment the control curves, graph I, are not quite normal. Thus the coronary resistance rises to an unusual height during systole as compared with the aortic pressure curve, and as a result the velocity curve indicates a very slow flow throughout systole. This was apparently due to the great reduction of venous return and cardiac output resulting from the various experimental procedures—ligation of both subclavian and the right common carotid arteries and compression of the lower end of the thoracic aorta.[1] The comparison of the curves of the control with those of arteriovenous fistula is however not invalidated by the variations noted in the control.

Graph II of figure 3 shows that opening the arterio-venous fistula has lowered the aortic diastolic pressure 25 mm. Hg without appreciably altering the systolic pressure and has very slightly lengthened the ejection period. The coronary resistance curve is altered much as it was in the case of aortic insufficiency: the systolic peak is lower, the curve falls to a lower level during diastole, it has not reached as great a height at the beginning of ejection and the beginning of the isometric rise does not precede the onset of ejection as much. These changes cause an alteration of the velocity curve resembling that produced during aortic insufficiency. The shaded areas show that the control $\frac{S}{D}$ ratio of $\frac{1}{2.16}$ is converted into a ratio of $\frac{1}{1.55}$ and that the area representing total flow per beat is reduced to approximately 82 per cent of the normal. Reduction of coronary flow is thus due solely to the lowering of aortic diastolic pressure and, as also occurred in uncompensated aortic insufficiency, the effect is mitigated by a reduction, especially during systole, of the resistance offered to coronary

[1] In effect this type of velocity curve is the exact opposite of that described by Gregg (9) following an increase in cardiac output produced by intravenous saline infusion.

Fig. 1. Diagram of a device called the coördirectograph, description in text.

Fig. 2. Curves illustrating the coronary flow under normal conditions I, during an uncompensated aortic insufficiency II, and during a compensated aortic insufficiency III: *A*—aortic pressure, *M*—mean blood pressure, *C*—coronary resistance (peripheral coronary pressure), *D*—velocity of flow (differential pressure curve). Shaded areas—volume flow with inscribed numbers indicating planimeter measurements of the areas on original enlarged graphs. Left hand ordinate—mm. Hg, aortic pressure and resistance curve. Right hand ordinate—mm. Hg, differential pressure curve. Abscissa—time, 0.2 second. Discussion in text.

Fig. 3. Curves showing the coronary flow under normal conditions I, and during an arterio-venous fistula II. Lettering same as figure 2. Discussion in text.

Fig. 4. Curves showing the coronary flow under normal conditions I, and during an aortic stenosis II. Lettering same as in figure 2. Discussion in text.

flow. The net result is a slight increase in systolic flow which is however not large enough to maintain the same flow per beat as the normal.

The effects of aortic stenosis. Five determinations of the effects of aortic stenosis on the coronary flow were made in two experiments. Typical results are illustrated in figure 4. The coronary resistance curve and the velocity curve of the normal, graph I, show the typical "normal" characteristics and the shaded areas representing flow show an $\frac{S}{D}$ ratio of $\frac{1}{1.96}$.

The aortic stenosis, graph II, has depressed the aortic systolic pressure somewhat, has elevated the aortic diastolic pressure slightly and has prolonged the ejection period. The coronary resistance curve, *D*, deviates from the normal in a manner opposite to that found in aortic insufficiency and arterio-venous fistula. Thus in comparison with the normal, I: it rises to a higher systolic peak, it falls to a lower diastolic level, the interval between the rise of the curve and the onset of ejection is lengthened and the curve is at a higher level at the onset of ejection. These changes in resistance are responsible for a steep isometric drop in the velocity curve which remains low throughout systole. With the relaxation of the ventricle the curve rises to a diastolic maximum practically equal to the normal. As a result, the shaded area representing systolic flow is reduced to $\frac{2}{3}$ its original value while that for diastole is reduced only slightly. The $\frac{S}{D}$ ratio is changed to $\frac{1}{2.56}$ and the total area representing flow per beat is reduced to approximately 82 per cent of the normal.

Thus, although mean aortic pressure remains essentially normal, a rather marked reduction in the coronary flow occurs. Compensation by peripheral constriction would help but little unless combined with slowing of the heart. The latter would reduce the minute work of the ventricle, and at the same time would increase the total coronary flow per minute because of an actual increase in the total time that the heart is in diastole per minute.

Discussion. It has been demonstrated that coronary volume flow per beat may decrease during each of the three lesions studied. Confirmation has been obtained for the observations of Smith, Miller and Graber (5) that the abrupt drop in aortic pressure during diastole is solely responsible for the decreased flow in uncompensated aortic insufficiency and arterio-venous fistula. In addition, evidence which could not be detected by previous methods is presented to demonstrate: that in uncompensated states of aortic insufficiency and arterio-venous fistula the decreased flow per beat is partly counterbalanced by an increased systolic flow, and that if the systolic pressure is elevated sufficiently to restore mean pressure to normal levels, the flow per beat may actually exceed the normal.

Since the reduction in flow during diastole, due to low diastolic pressure,

may thus be compensated to varying degrees by an increase in systolic flow through elevation of systolic pressure, systolic aortic pressure assumes great importance. Although there is at present no direct evidence that the increased flow accomplished by such compensation would be commensurate with that demanded by the increased work of the heart, such a result might be inferred from the facts that the hearts do continue to beat during a severe aortic insufficiency, and that they may even increase their vigor sufficiently to compensate for the fall in diastolic pressure without the aid of mechanical peripheral constriction.

The conception that the coronary arterial system might offer varying degrees of resistance to flow has been generally recognized. Most observers, misled by the false conception that flow does not occur in systole have, however, not clearly understood this second factor controlling coronary flow. These experiments clearly demonstrate that the changing state of myocardial resistance to flow during *both systole and diastole* is equally as important as aortic pressure in determining coronary flow.

The question next arises whether such alterations in intramural resistance are myocardial or vascular in origin. While an answer must be deferred until more information is available, it is advantageous to summarize the experimental data available and see how they fit different possible explanations.

In all of the studies made in this Laboratory ((6) (9) and the experiments reported in this paper) the systolic peripheral coronary resistance has varied in general as the systolic aortic pressure and therefore also as the systolic intraventricular pressure, although no exact parallelism exists. It is not surprising therefore that the systolic peripheral coronary resistance should have elevated considerably during aortic stenosis when the systolic intraventricular pressure rose markedly despite the absence of any elevation of aortic pressure.

The fact that the ratios of the systolic peripheral coronary resistance to the systolic aortic pressure during aortic insufficiency and arterio-venous fistula did not remain the same as the ratios in the normal controls is not so easily explained. These results and also those reported by Gregg (9) indicate that, in the dog under the conditions of these experiments, increased diastolic size of the ventricle and a wide pulse pressure, produced by augmented venous return, tend to decrease the ratio, while decreased venous return has the opposite effect, even when there is no change in the level of aortic systolic pressure. This finding might be interpretated to mean that the vessels are mechanically opened by distention of the heart. Such an interpretation cannot be accepted however until the relative effects of isotonic and isometric contraction on coronary resistance are more clearly understood. Furthermore some doubt about the operation of such a mechanical factor arises from a study of records obtained during large

beats following premature ventricular systoles.[2] In these large beats the diastolic size and pulse pressure are momentarily increased without significantly changing the ratio of systolic peripheral coronary resistance to systolic aortic pressure.

It was hoped that a study of the contour changes of the resistance curves might help to clarify the cause of the changes noted in the systolic and diastolic levels of the resistance curves. A survey of all records, including those published in this paper, shows that while the contour of the peripheral coronary pressure curves varies somewhat in different hearts, in any one animal it remains reasonably constant under widely different circulatory conditions. The rate of rise and fall alters but little, the rise proceeds in about the same manner, despite variations in the isometric period, and the point on the rise at which ejection starts, and the inflection of the curve marking the end of the rapid decrease in resistance in early diastole occur at practically the same points. Such curves therefore seem to offer little evidence that variations in the mode of contraction and relaxation under grossly different dynamic conditions play a large part in determining the extremes of resistance developed during the cardiac cycle.

While it is conceivable that minor changes of resistance might be due to variable collateral transmission of pressure under different aortic pressure heads, results presented previously (10) seem to indicate that such transmission cannot explain the major changes noted in these experiments.

Consideration of the extent to which changes in caliber of coronary vessels may possibly be concerned cannotbe omitted. Such changes might be caused by passive distention due to different extra-arterial tension or to active constriction or dilatation through chemical or nervous action. Such nervous actions are conceivable in the preparation used, since the manner of incising the epicardium longitudinally in isolating the coronary ramus should leave most of the vasomotor nerves intact. Speculations about how such vascular changes could effect the relation between aortic pressure and intramural resistance during systole would, however, be hazardous in the present state of available experimental facts.

SUMMARY

Since circulatory abnormalities such as aortic insufficiency, aortic stenosis and arterio-venous fistula produce striking changes in the heart and circulation and occasionally myocardial injury, knowledge of their effects on coronary circulation has considerable clinical and physiological signif-

[2] During these experiments there were observed, immediately following premature ventricular systoles, twenty instances of large beats in which the aortic systolic pressure closely approximated that of the preceding and succeeding normal beats and in which it was possible to determine the peripheral coronary resistance during systole. For the sake of economy of space illustrative records are omitted.

icance. The effects of these abnormalities on coronary circulation have therefore been studied in the dog by a slight modification of the method previously reported by Green, Gregg and Wiggers (6).

These studies revealed that under the conditions of these experiments: 1, lesions of the type of uncompensated aortic insufficiency and arteriovenous fistula cause a decrease in coronary flow chiefly during diastole, by lowering aortic diastolic pressure; 2, this decreased flow is, to a considerable extent, mitigated by a concomitant increase in the coronary flow during systole resulting from a relative lowering of the systolic peripheral coronary resistance in relation to aortic pressure; 3, in these lesions, compensation through peripheral vascular constriction, sufficient to restore a normal mean blood pressure, may increase coronary flow to or even above normal because the systolic peripheral coronary resistance is elevated relatively less than the aortic pressure, and 4, lesions such as aortic stenosis decrease coronary flow mainly during systole by causing a relatively higher degree of systolic peripheral coronary resistance in relation to aortic pressure.

The question is raised whether an increased flow, such as that obtained by compensation during aortic insufficiency, is commensurate with the increased work of the heart.

It is concluded that the increased systolic peripheral coronary resistance in aortic stenosis is caused by the abnormal height of intraventricular systolic pressure compared with aortic systolic pressure. Some of the factors considered in the attempt to explain the other changes of peripheral resistance observed are, that the diastolic size of the ventricle and the wide pulse pressure operate in some manner to alter the extravascular constriction of the vessels and that reflex or metabolic action may cause changes in vascular size. It is considered impossible, however, at the present time to arrive at any definite conclusions.

The author wishes to express his appreciation for the guidance of Prof. Carl J. Wiggers.

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THE EFFECT OF ATROPINE AND PILOCARPINE UPON THE EMPTYING TIME OF THE HUMAN STOMACH

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Experimental evidence from a variety of sources has demonstrated the important rôle played by the central nervous system in gastro-intestinal motility. This concept is too recent an acquisition to have reached the textbooks of physiology (1). Electrical stimulation of the vagi in experimental animals has been demonstrated to cause an increase or decrease in gastric tonus depending upon the existent state of tonus (Carlson and Pearcy, 2; McCrea, McSwiney and Stopford, 3). Section of the vagi in cats and dogs has resulted in a marked prolongation of gastric emptying time (McCrea, McSwiney and Stopford, 4; Fetter, Barron and Carlson, 5; Meek and Herrin, 6). The last mentioned workers emphasized that the marked lack of gastric tonus after vagotomy was the principal cause of the delayed emptying. The effect of insulin in stimulating gastric motility in both dog and man has been demonstrated to depend upon the vagus nerve, atropine and vagotomy abolishing this effect (Quigley and Carlson, 7; Quigley, Johnson and Solomon, 8; Wilder and Schultz, 9). Electrical stimulation of the tuber region of the hypothalamus in chloralosed cats has been found to result in increased intragastric pressure and peristalsis. This motor response was absent after vagotomy (Beattie and Sheehan, 10). Electrical stimulation of the lateral hypothalamus in unanesthetized cats was reported by Kabat, Anson, Magoun and Ranson (11) to result in cessation of peristalsis and loss of tone in the stomach and intestine. This result they attributed to stimulation of a center for sympathetic activity. Electrical stimulation of the cerebral cortex in dogs and monkeys had no effect on gastric motility according to May (12) but Sheehan (13) has reported that cooling or warming the cortex would result in decreased or increased activity of the digesting stomach. Watts and Fulton (14) found that faradic stimulation of most parts of the pre-motor area of monkeys caused vigorous motor activity of the intestines. Also, several monkeys died with intussusception after removal of the pre-motor area. Finally, after an extensive study of the mechanism of gastric evacuation Thomas and his associates (15) have concluded that two reflexes, with their receptors in the duodenum, are involved in gastric emptying and that the one,

whose pathway is in the central nervous system, regulates gastric emptying to a greater extent than has formerly been supposed. In this report we have determined the effect of atropine and pilocarpine upon the emptying time of the human stomach with the expectation of demonstrating the rôle of the vagus nerve in the gastric emptying mechanism of the human subject.

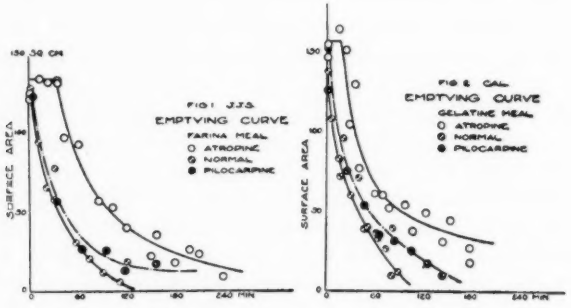
The effect of atropine upon gastric secretion and motility has been very extensively reviewed by Altshuler (16). The effect of atropine, as observed by most students of this problem, has been inhibition of peristalsis and retarded emptying. Ötvös (17) using a barium meal and roentgenological examination found that atropine delayed gastric emptying in 29 of 56 clinical cases. He thought the delay was due to pylorospasm. Löwy and Tezner (18) administered a gruel and barium sulfate to 83 children, who ranged in age from 5 to 14 years. They reported that 1 mgm. of atropine subcutaneously delayed gastric emptying in 78 per cent of the cases and that 2 mgm. delayed it in all cases. However their report contains the surprising statement that atropine is without effect on gastric emptying in adults.

The results with pilocarpine are contradictory. Horne, McDougall and Magee (19) administered sugar solution to rats by stomach tube. Upon sacrificing the animals, those receiving pilocarpine were found to have less residual sugar in their stomachs than the normals. Bennett (20) found by aspiration of gastric contents, that pilocarpine delayed the emptying of a gruel meal in medical students. Using a similar procedure, Ferguson, McGavran and Smith (21) found that pilocarpine promoted gastric emptying in monkeys. Löwy and Tezner (18) in their series of 83 children found that 50 per cent of their cases had increased emptying time with 5 mgm. of pilocarpine and all had prolonged emptying with a 10 mgm. dose.

EXPERIMENTAL PROCEDURE. The effect of atropine and pilocarpine on the gastric emptying time was studied on 13 healthy human subjects. With one exception these were first and second year medical students. On the day of the test a light breakfast was taken at the usual time and the barium meal was taken at noon. With the idea of testing the factor of consistency two different meals were used. The farina meal, which had a thick consistency, consisted of 30 grams of farina cooked in 300 cc. of water to which were added 150 grams of $BaSO_4$. The gelatin meal which was of such liquid consistency as to permit drinking, consisted of 30 grams of jello gelatin in 300 cc. of water plus 150 grams $BaSO_4$. The tests were made in successive rotations of normal, atropine and pilocarpine in order to give comparable results throughout the year.

Aqueous solutions of the drugs were injected subcutaneously. The dosage of atropine sulfate varied from 0.2 mgm. to 1.2 mgm. and that of pilocarpine hydrochloride from 5.0 to 10.0 mgm. per subject. The effects

of the drugs were always very pronounced before the meal was ingested. The emptying of the stomach was determined by fluoroscopic examination. The initial emptying was taken as the interval between the beginnings of the meal and the first appearance of barium in the intestine. The rate of emptying was studied by tracing the stomach shadow as projected on the fluoroscopic screen. The first tracing was taken directly after initial emptying had begun and therefore it is not a factor in the shape of the curve. Care was exercised to make the tracing under the same conditions each time. The area of each tracing was determined by means of a planimeter and the area plotted as the ordinate and time as the abscissa. In several cases the frequency of the gastric peristaltic waves was determined. Representative emptying curves for the two types of test meal are shown in figures 1 and 2. The data for two complete series are plotted in each case.



Results. The normal emptying time gave rather consistent values over a period of months. The solid farina meal and the liquid gelatin meal gave similar results, the difference being only one of degree. The effect of atropine in delaying gastric emptying was much greater with the solid meal, table 1, than with the liquid one, table 2. Atropine increased the initial emptying time in 6 of the 8 subjects taking farina 60 to 436 per cent and in 3 of the 5 subjects taking the gelatin meal 57 to 222 per cent. The final emptying time was increased 40 to 154 per cent in the 8 subjects ingesting farina and 4 to 95 per cent in the 5 subjects taking the gelatin meal before the injection of atropine. A 0.2 mgm. dose of atropine was tried in 6 subjects and had no appreciable effect on the emptying time of 4, but resulted in a 15 to 61 per cent increase in the remaining two subjects.

Pilocarpine decreased the initial emptying time in 5 of the 8 subjects taking farina 11 to 70 per cent, had no effect in one and markedly increased it in another. It shortened the initial emptying 16 to 25 per cent in 2 of the 5 subjects taking gelatin. Pilocarpine increased the final emptying time in

TABLE 1

Effect of atropine and pilocarpine on gastric emptying time with a farina meal

SUBJECT	NORMAL		ATROPINE			PILOCARPINE			
	Emptying		Pulse change per minute	Emptying		Dosage	Pulse change per minute	Emptying	
	Initial	Final		Initial	Final			Initial	Final
	minutes	*minutes*		*minutes*	*minutes*	*mgm.*		*minutes*	*minutes*
E. A. B.	4	93	60–100	2.45	175	7	68–80	1	93
	3	91	62–112	4.5	130	10	70–80	2	137
	4	106	68–104	7.0	145	5		1	110
Av.	3.6	97		4.6	150			1.3	113
G. R. H.	2	84	58–86	3.5	178	7	68–84	2	105
	2	77	56–96	4	92	10	68–84	2.5	164
	2	81	56–80	3	96	5	64–80	1.5	66
Av.	2.0	81		3.5	122			2.0	112
J. F. M.	3	114	76–118	8	245	7	88–92	3.0	122
	9	134	72–88	3.5	110	10	80–96	2.0	104
	3	99	80–120	12.5	183	5		3.0	99
Av.	5.0	116		8.0	179			2.7	108
A. L. R	2	118	76–100	10.6	270	7	88–88	11.0	144
	7	119	71–92	6.5	225	10	102–111	2.5	80
	9.5	107	92–100	29.5	273	5	84–96	3.0	104
Av.	6.2	115		47.3	256			5.5	109
J. J. S.	7	150	80–114		214		90–101		190
	6	107	96–116		275	10	80–92	5	219
	2.5	92	90–112	10	233	5	88–100	3	184
			90–114	19.5	240				
Av.	5.2	116		14.7	240			4.0	198
P. W. S.	11	142	68–108	18	270	7	78–84	4	112
	3	75	72–112	17	300	10	76–86	2	203
	8	115	76–108	22	275	5	76–88	4	133
Av.	7.3	111		19.0	282			3.3	149
R. C. H.	3	111	64–134	27	215	7	56–64	26	182
	4	124	60–98	15	216	10	68–78	4	248
	4	113	66–120	16	214	5	60–60	5.5	164
Av.	3.6	116		19.3	215			11.8	198
M. B.	4	128	63–87	4	238	7	70–84	26	174
	17	152	66–80	5	193	10	60–84	5	159
	4	113	66–104	6	139	5	66–74	2	270
Av.	8.3	131		5.0	190			11.0	201

10 of the 13 subjects 5 to 71 per cent and shortened it immaterially in the others. Thus we see that both drugs have influenced the normal emptying of the stomach. Atropine has markedly hindered the normal emptying. Pilocarpine has favored initial emptying but has not favored the evacuation of the last portion of the gastric contents.

Discussion. The atropinized human stomach showed a great lack of tonus. It was irregular in outline as though passively fitting itself into the crevices of the adjacent viscera. For considerable time post-prandially, it always contained a large air space and the contents could be made to splash around by pushing the subject's abdomen. Its projection on the fluoroscopic screen resembled very much that of the vagotomized dog's stomach. Peristaltic waves passed over the atropinized stomach at a normal frequency of about 4 per minute. The strength of peristalsis was probably reduced since it very likely parallels the degree of tonus under most physiological conditions. Although some of the gastric atony might be due to the action of atropine on the gastric musculature it more likely is due to a blockade of the impulses which continually come down over the motor vagus to maintain normal gastric tonus.

Atropine lengthened gastric emptying time. The degree of lengthening corresponded to the length of the normal emptying time. This is best illustrated by subjects A. L. R. and P. W. S. in table 1 and C. R. L. of table 2. The total correlation coefficient between normal final emptying time and the per cent lengthening of final emptying time resulting from atropine administration was calculated to be -0.482. The statistical probability that this correlation coefficient expresses only an accidental relationship is one chance in about fifteen. These results suggest very strongly that gastric emptying time may be an indication of gastric vagal tone just as the pulse rate is an indication of cardiac vagal tone.

The gastric emptying curves in figures 1 and 2 show that there are two sites of delay, one at the beginning and one occupying the last third of the emptying time. The initial delay in the emptying and the prolonged initial emptying time might be due to a failure of the stimulus of a filled stomach to reflexly affect the gastric musculature because of the atropine blockade of the motor fibers in the intrinsic plexus or it might be due to a lack of vagal gastric tonus. That these premises were true was seen when five subjects ingested a barium-gelatin meal 50 per cent larger than normal. The results were consistent and the curves of emptying showed no initial delay, the gastric evacuation starting promptly and proceeding very rapidly.

Following the initial delay, the rate of emptying proceeds about the same as in the normal. In attempting to understand this response, one needs to remember the demonstration of Loewi and Navratil (22) that atropine paralyzes the vagus not by preventing the liberation of acetyl choline but

by blocking the excitation of the motor effector by acetyl choline. One might consider that during the initial delay the discharge of nervous impulses by receptors in the gastric mucosa has resulted in the continual production of acetyl choline in the stomach as has been demonstrated by

TABLE 2

Effect of atropine and pilocarpine on gastric emptying time with gelatin meal

SUBJECT	NORMAL		ATROPINE				PILOCARPINE			
	Emptying		Dosage	Pulse change per minute	Emptying		Dosage	Pulse change per minute	Emptying	
	Initial	Final			Initial	Final			Initial	Final
	minutes	*minutes*	*mgm.*		*minutes*	*minutes*	*mgm.*		*minutes*	*minutes*
H. C. M.	1.25	118	1.2	72–120	0.75	192	5	72–84	0.5	145
	0.5	115	1.2	78–96	13.0	227	10	72–88	2	161
	1.3	90	1.2	72–96	2.5	233†	7	72–76	0.75	96
Av.	1.0	108			5.4	217			1.1	134
			0.2	72–78	1.5	98				
R. J. R.	0.75	85	1.2	81–100	0.5	207	5	72–94	0.5	194
	0.5	123	1.2	81–112	8.0	217	10	88–104	1.5	103
	3.5	77	1.2	80–100	1.5	246†	7	85–86	1.5	98
Av.	1.6	95			3.3	223			1.2	132
			0.2	78–96	0.5	106				
C. H. R.	1.25	144	1.2	80–124	1.5	189	5	72–84	2	205
	2	197	1.2	84–128	1.0	199	10	68–88	6	122
	1.0	96	1.2		3.0	236†	7	68–88	1.0	95
Av.	1.4	136			1.8	208			3.0	141
			0.2	76–60	2.0	156				
W. H. P.	2.75	109	1.2	72–120	1.5	197	5	72–100	1.5	178
	3.0	128	1.2	86–100	4.0	206	10	80–88	3.0	87
	1.5	94	1.2	76–82	4.0	211†	7	76–88	1.5	173
Av.	2.6	110			3.1	204			2.0	146
			0.2	60–76	1.0	178				
C. R. L.	1.0	142	1.2	90–130	2.8	184	5	84–104	0.75	172
	0.75	210	1.2	76–96*	22.0	197	10	74–104*	1.5	180
Av.	0.9	176							1.1	176
	0.5*	96								
	2.0*	105								

* Determinations were made in October, 1935.

† Meal was 50 per cent larger.

Dale and Feldberg (23) to occur under vagal stimulation. Finally, a point is reached when the concentration of acetyl choline at the muscle is sufficient to break through the atropine blockade and thereafter gastric evacuation proceeds at a normal rate.

The final delay in emptying of the atropinized stomach is undoubtedly due to a lack of gastric tonus. Tonic shortening of the muscle fibers has not kept pace with evacuation of the gastric contents. It could hardly be due to Thomas' enterogastric reflex because the atropine would paralyze the vagal inhibitory fibers. It is interesting to note that another example of this sequence, initial delay, normal rate of rapid emptying and terminal delay in the gastric emptying curve is seen in the study of Hellebrandt and Tepper (24) on the effect of severe exercise upon gastric emptying in the human subject. The similarity of their emptying curves to those of the atropinized stomach suggests that a lack of vagal tonus in the stomach may be the cause of the delays in their experiments and one of the physiological situations in which this lack of vagal tonus in the stomach can be brought about.

Pilocarpine appeared to increase the tonus of the stomach. Its outline was smooth and regular, the incisura was accentuated and the air bubble was very small or absent. The shortened initial emptying time and the emptying curves for pilocarpine in figures 1 and 2 show that pilocarpine favors gastric evacuation until about two-thirds of the contents are discharged. The increased tonus of the stomach caused by the pilocarpine is undoubtedly the principal factor in promoting this emptying. However, pilocarpine in all cases lengthened final emptying time and the emptying curves show that the delay occurs near the end. This delay is very likely due to a reinforcement of Thomas' enterogastric reflex. Pilocarpine would be expected to stimulate not only the motor vagus to the stomach but the vagal inhibitory fibers as well and the demonstrations of Doyon (25) and Battelli (26) that pilocarpine initially causes inhibition of the stomach would seem to support this idea. This inhibitory action of pilocarpine in the stomach summated with the normal reflex inhibition to gastric motility of a filled intestine has caused delay in the completion of evacuation. The purposefulness of the entero-gastric reflex in the prevention of an excessively rapid discharge of chyme into the intestine is quite evident. The favorable effect of pilocarpine upon gastric motility might be of some use in clinical cases of gastric atony.

The 5 mgm. dose of pilocarpine accelerated gastric evacuation whereas the 10 mgm. dose seemed to produce an actual inhibition to gastric motility. For a few minutes after ingestion of the meal very few or no peristaltic waves were seen. This inhibitory effect is expressed in the longer initial emptying time with the 10 mgm. dose than with the 5 mgm. in all of the subjects in table 2 and in a longer final emptying time with the 10 mgm. dose in E. A. B., G. R. H., J. J. S., and M. B. of table 1. It is to be noted that Cushing (27) found that a 2.5 mgm. dose of pilocarpine injected into the cerebral ventricles of clinical patients promoted gastric peristalsis, whereas a 12 mgm. dose intramuscularly caused pyloro-spasm, retrograde

peristalsis and vomiting. The difference in response he attributed to a central action in the case of ventricular injection and a peripheral action with the intramuscular injection. However, in our experience these two dosages injected subcutaneously would produce at least temporarily motor and inhibitory effects. The effect of the larger dose we attribute to a preponderance of stimulated inhibitory fibers in the vagus over the motor fibers.

As seen in the tables, pilocarpine injection resulted in marked acceleration of the pulse in all subjects, although several had a very transient slowing initially. This observation is in accord with those reported by Blomberg and Rönnell (28) in their extensive review of this subject. They concluded from their survey that systolic blood pressure showed no great change with pilocarpine, either increasing or decreasing slightly. The means by which pilocarpine accelerates the pulse is not known but it has been suggested to be due to its action on the medullary centers.

SUMMARY

Atropine increases both initial emptying and final emptying times of the normal human stomach. The lengthening of final emptying time is due to two delays in evacuation. One occurs during the first half hour after the meal and the other occurs near the end when the volume of gastric contents has become greatly reduced. Between these two delays gastric evacuation proceeds at a normal rate. These observations are interpreted as a demonstration of the importance of the vagus nerve in maintaining that gastric tonus and peristalsis necessary for a normal emptying of the human stomach. The length of the final emptying time seems to be an indication of the degree of gastric vagal tone.

Pilocarpine in most of the subjects favored initial emptying. However in 10 of the 13 subjects final emptying time was increased from a slight to a moderate degree. For about two-thirds of the emptying, the rate with pilocarpine is as rapid or faster than the normal. The delay in evacuation comes only near the end. The favorable effect of pilocarpine on gastric evacuation demonstrates the value of strong gastric tonus and peristalsis in the mechanism of emptying and how these two factors can be heightened by vagal activity. The delay in the completion of evacuation may well be considered as evidence of Thomas' enterogastric reflex. These studies with atropine and pilocarpine have furnished considerable evidence for the important rôle of the vagus nerve in the normal mechanism of gastric emptying in the human.

To the medical students who willingly coöperated in this study I wish to express my full appreciation.

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THE EFFECT OF ATROPINE AND PILOCARPINE UPON GASTRIC EMPTYING IN NORMAL AND DENERVATED DOGS

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In the preceding paper (1) it was reported that both atropine and pilocarpine increased the emptying time of the human stomach. With atropine, delayed evacuation occurred at the beginning and again near the end with a normal rate of emptying interposed between the delays. It was suggested that the delays were due to a partial paralysis of the gastric vagus. With pilocarpine, the delayed evacuation occurred only near the end and this delay was attributed to accentuation of Thomas' entero-gastric reflex (2).

The limitations of human experimentation on this problem demonstrated the need for animal studies. It was conceivable that there were three loci on which these drugs could exercise their effects, namely, the gastric musculature, Auerbach's plexus and the endings of the vagus nerve. In this connection, the studies of Gasser (3) upon isolated smooth muscle preparations from cats' intestine are particularly pertinent. Using strips of circular muscle, histologically free of nerve cells, he tested the effect of atropine and pilocarpine as well as other alkaloids and bases upon the motility of these denervated muscle strips. Atropine inhibited the rhythmic contractions and pilocarpine in one preparation stimulated the muscle and in two others had no effect. From these results and those obtained with the other drugs Gasser concluded that the drugs acted directly upon the muscle fibers and not upon the plexus. His results show that atropine and pilocarpine may directly affect smooth muscle of the gastro-intestinal tract but the conclusion that the plexus is not concerned is very much open to question. The absence of the nerve cells in these preparations is not proof that they are less susceptible to the action of atropine and pilocarpine than the corresponding muscle fibers. One might expect the nerve cells of Auerbach's plexus to possess greater automaticity and rhythmicity than the smooth muscle fibers which they innervate and that the nerve cells would be more susceptible to atropine and pilocarpine than the muscle fibers. Gasser's results with pilocarpine indicate that this expectation may hold. Furthermore, gastric peristalsis is a more com-

plicated movement than merely smooth muscle contraction. In the absence, then, of conclusive evidence, it would seem that we might accept the proposition that these two drugs exert an effect on the smooth muscle directly, as well as on the endings of the post-ganglionic nerve fibers. Furthermore, it would seem that any marked difference between the results obtained with these two drugs in the normal dogs and the denervated ones could be attributed to the extrinsic nerves. Likewise the difference between the results on the denervated dogs and human subjects might be attributed to the extrinsic nerves in the latter.

Although Brown and McSwiney (4) using isolated strips from rabbits' stomach found that pilocarpine was excitatory and atropine inhibitory to all regions of the stomach, a survey of the literature revealed no study of the effect of atropine and pilocarpine upon the emptying of a denervated stomach. Such a study is the basis of this report.

EXPERIMENTAL PROCEDURE. Healthy dogs whose body weights ranged from 8.2 to 18.2 kilos were used in this study. As indicated in table 1 three were normal and the others had certain denervations. Dogs 4 and 5 had been vagotomized 20 months and sympathectomized 14 months. Dogs 6 and 7 had been vagotomized and sympathectomized 2.5 months and dog 8 for 2 weeks. Dogs 9, 10 and 11 had been vagotomized 2 weeks. In addition to vagotomy, dog 10 had a left-sided sympathectomy. The experimental studies with these dogs extended over a period of about 3 months. The sympathectomy consisted of excision of the lumbar chains and section of the splanchnic nerves and the vagotomy consisted of section of the vagi just cephalad to the diaphragm.

The meal consisted of 12.5 grams of milk-soaked Champion dog biscuit and 4.1 grams $BaSO_4$ per kilo of body weight. This meal, although soft, was not a gruel so that its evacuation was a good test of the motor activity of the stomach. Since the stomachs of vagotomized dogs practically always retain some food even after a moderate fast the dogs were given 1 mgm. of apomorphine about 9 hours before the barium meal.

The period of initial emptying was taken as the interval between the initial ingestion of food and the first discharge of food into the intestine. The rate of emptying was determined by plotting a curve with time on the abscissa and the cross-sectional area of the stomach as projected on the fluoroscopic screen on the ordinate. To minimize the unavoidable error involved in tracing the stomach shadow a standard routine was always followed. Since the vagotomized dogs had such a long final emptying time, exceeding 15 to 24 hours, the emptying curve was considered of greater value in ascertaining the effect of these drugs. In all dogs except no. 3 which had 2 complete series of determinations, we made at least 3 trials with each normal, atropine and pilocarpine. In some cases 4 or 5 determinations were made. The data in table 1 are averages of at least 3

determinations in all dogs except no. 3. The curves in figures 1, 2, 3 and 4 are plotted with the data from 2 determinations of each of normal, atropine and pilocarpine.

The drugs were dissolved in water and injected subcutaneously. Inasmuch as the dogs refused to eat when the effects of the drugs were pronounced, the injections were made only a few minutes before the meal was presented to the dog. For this reason the effects of the drugs on initial emptying are minimal rather than maximal. On the basis of Sollmann's (5) *Manual of Pharmacology* the dosage of atropine sulfate was taken at 0.03

TABLE 1

Average emptying time in normal and denervated dogs

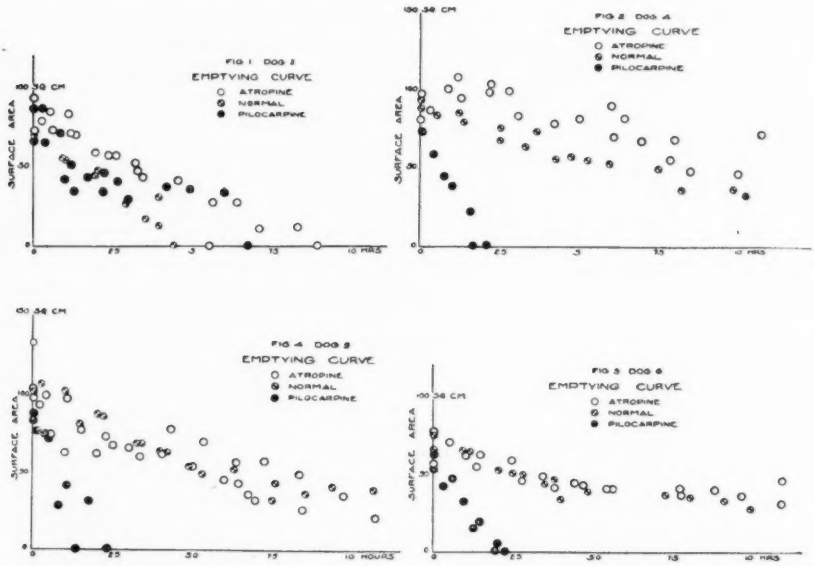
DOG NUMBER	CONDITION	INITIAL EMPTYING			FINAL EMPTYING		
		Normal	Atropine	Pilocarpine	Normal	Atropine	Pilocarpine
		min.	*min.*	*min.*	*min.*	*min.*	*min.*
1	Normal	5.6	11.6	5.0	277	>514	209
2	Normal	5.6	11.8	4.3	196	>476	288
3	Normal	8.0	59.0	14.0	269	317	347
4	Denervated	7.5	16.0	4.3	>599	>819	146
5	Denervated	11.0	26.3	3.5	>634	>820	137
6	Denervated	21.3	25.5	4.0	>652	>858	<132
7	Denervated	14.2	18.3	3.5	>678	>858	188
8	Denervated	>29.0	38.0	8.0	>777	>801	126
9	Vagotomy	22.0	41.3	16.0	>713	>819	184
					fine biscuit		87
					meat		25
10	Vagotomy	23.0	40.8	8.0	653	654	304
					fine biscuit		218
					meat		222
11	Vagotomy			12.0			182
				11.0	fine biscuit		99
					meat		24

mgm. and pilocarpine hydrochloride at 0.4 mgm. per kilo of body weight. In the case of atropine a second injection was given about 6 hours after the first. These dosages did not cause extreme changes. Ordinarily there was no vomiting with pilocarpine.

RESULTS. The three normal dogs of table 1 gave results very similar to those obtained with the human subjects. Atropine increased the average initial emptying time 107 to 637 per cent and the average final emptying time 15 to 146 per cent. The atropinized dog's stomach showed delayed emptying as seen in figure 1, initially. The initial delay in the emptying curve for the dogs does not begin directly after the feeding because the full

effect of atropine was not yet manifest. (The dogs would not eat when the signs of atropine were marked.) This also explains why the volume of the stomach is larger on the second and third examinations than on the first.

Pilocarpine had a slight tendency to shorten the initial emptying time and in dog 1 shortened the final emptying time but increased it in the other two dogs. In two of the three dogs, pilocarpine favored gastric evacuation until the last third of the emptying period at which time delayed emptying was apparent. In the third dog pilocarpine had no favorable effect on emptying.



The denervated dogs (table 1) in the normal emptying trials demonstrated the inadequacy of the emptying mechanism in the vagotomized dog's stomach as has been previously reported. The average initial emptying time for all the denervated dogs was 17.3 minutes as compared to 6.4 minutes for the normal animals and for final emptying time the difference is much greater since the average for normal dogs was 247 minutes and in the denervated exceeded 669 minutes.

In the denervated dogs the two drugs produced consistent results. Atropine increased the average initial emptying time in all dogs 132 per cent and pilocarpine decreased the average initial emptying time 61 per cent. However, the really striking motor response to pilocarpine was seen in the

completely denervated dogs, in whom the prolonged final emptying time was shortened to a value in all cases much less than the normal emptying time of normal dogs (table 1). The emptying curves of these animals showed no delay. On the other hand, the dogs with only sympathetic innervation (nos. 9, 10 and 11) did not show as short an emptying time with pilocarpine as the completely denervated. With the biscuit meal of the usual consistency, the emptying time with pilocarpine remained between 3 to 5 hours. Barium meals of the same weight in the form of either milk-soaked biscuit broken to pieces of the size of rice kernels or hamburger were evacuated in much less time. A meal consisting of 75 grams of egg yolk, 15 grams of $BaSO_4$ and 100 cc. of water was evacuated in less than 30 minutes.

Discussion. The similarity of the results (table 1) for normal emptying in the denervated dogs and for emptying in the atropinized normal dogs is convincing evidence of the rôle of the vagus in the gastric emptying mechanism. The effect of atropine in the denervated dogs seemed to indicate the amount of gastric tonus recovered since the vagotomy. This is best represented, in the case of initial emptying, by dogs four and seven. In the emptying curves in figure 2 the volume of the atropinized stomach is greater than that of the normal (20 months post-vagotomy). In figure 3 (3 months post-vagotomy) and in figure 4 (1 month post-vagotomy) the normal and atropine curves of emptying are close together. These date indicate that the recovery of gastric tonus in vagotomized dogs may not ba nearly as rapid as has been reported (6).

Atropine apparently had very little effect on the rate of gastric evacuation in the denervated dogs, inasmuch as the slope of the emptying curves for atropinized stomach is about the same as for the normal. This seemed to hold for all of the denervated dogs except, after 9 hours post-prandially in dog 9, atropine slowed up the emptying. This observation being true, the effect of atropine in delaying gastric evacuation in the normal is due to a paralysis of the vagus nerves to the stomach and not to its action on Auerbach's plexus or the gastric musculature, since these last two factors are common to both types of animals. Furthermore, this deduction can be extended to the studies of the effect of atropine on gastric evacuation in the human subjects and emphasizes the rôle of the vagus in the emptying mechanism of the human stomach.

Pilocarpine in the denervated dogs shortened final emptying time in a striking fashion to a value about half that of normal dogs (table 1). Reference to the emptying curves in figures 2, 3 and 4 shows that in the completely denervated dogs there is no terminal delay. The stomach continued to pour out its crude contents even though the duodenum was very much distended. In many cases these dogs would pass in their feces com-

pletely undigested biscuit. Egg yolk would appear in the feces within 30 minutes after being placed in the stomach. The above conditions we could not duplicate in the normally innervated dogs.

However, dogs 9, 10 and 11, possessing sympathetic innervation, did not always show a rapid emptying with pilocarpine. Their final emptying time while greatly shortened still remained about that of normal emptying in a normal dog. Consistency of the meal seemed to be of more importance in these dogs. The failure of pilocarpine to shorten emptying time to less than 2 hours in these animals could hardly be due to secreted adrenalin (Sollmann, 5a). Moreover, the meals of finer consistency did result in a decidedly sub-normal emptying time. Part of the failure of dog 10 to give as short emptying time as dogs 9 and 11 with the meals of fine consistency is explained by the autopsy finding that one strand of the vagus to the stomach had been missed at the vagotomy. The observation that the sympathetic nerves tend to slow up the passage of chyle through the intestine is very old (Cannon, 7). In the course of other studies we have lost dogs because of intussception after abdominal sympathectomy. Furthermore, in our sympathetically innervated dogs although pilocarpine caused meals of fine biscuit, ground meat and egg yolk to leave the stomach in a very short time, it never caused these animals to pass undigested food in the feces as it did in the completely denervated dogs. It is significant in this connection that pilocarpine has been found to have very little effect on the propulsive motility of jejunal loops in normal dogs (8).

The response of the stomach to pilocarpine in these studies makes possible certain deductions concerning its mechanism of emptying. A comparison of the striking effect of pilocarpine upon the gastric emptying time in the denervated dogs with its effect in the normal dogs and human subjects constitutes a demonstration of Thomas' entero-gastric reflex. The complete failure of the pyloric sphincter, with its intrinsic nerve supply to properly regulate gastric evacuation is herewith demonstrated. Thomas and his co-workers (2) have pointed out that pilocarpine abolished the residual inhibitory reflex in vagotomized dogs. Perhaps this abolition is due to the dominating motor action of pilocarpine. However, in the normal animals the vagal reflex is sufficient to hold back the stomach even though it is driven by large doses or repeated doses of pilocarpine. The vagal reflex functions adequately even with the meal of egg yolk which was selected not because of its demonstrated chalone action on gastric motility but because of its physical homogeneity, its semi-fluidity and its native biological nature. Such a meal should be a severe test of the regulating reflexes of gastric emptying. Twelve trials with this meal and pilocarpine in 5 normal dogs gave the following results. In 3 trials the dogs emptied in 35 minutes or less and in the others the dogs required an hour to 3.5 hours. Finally the very rapid emptying of the denervated stomach under

the influence of pilocarpine demonstrates that within the stomach itself there is an adequate mechanism for carrying out the coördinated movement of gastric peristalsis and emptying. The additional equipment required for normal emptying is the driving force which comes from the central nervous system over the vagus nerve to maintain the proper degree of gastric tonus and strength of peristalsis and the regulating reflex from the intestine via the vagus to adapt the rate of emptying to the capacity of the intestine. Thus, it is seen in these studies that the gastric vagus, although it may function under special conditions such as suggested by Cannon's first report (9) of the effect of the emotions upon gastro-intestinal motility, actually functions by its tonic and reflex activity as an integral part of the normal mechanism of gastric emptying.

Reflex stimulation of the gastric vagus generally results in inhibition (2) (10). In attempting to demonstrate inhibitory post-ganglionic fibers to the stomach we administered 4 to 6 egg yolks about 1.5 hours before the meal to produce a partial state of inhibition in the stomach (11). In the denervated and normal stomach the egg yolk delayed gastric evacuation. Small doses of pilocarpine (about 0.1 mgm. per kilo) accelerated gastric evacuation but when combined with the egg yolk and biscuit meal we sometimes obtained marked inhibition to gastric motility. The number of peristaltic waves was reduced from 14 to 6 per minute. The emptying curve flattened out. Once in a normal and again in a completely denervated stomach there seemed to be no gastric motility at all and in about 30 minutes complete emesis occurred. However, our difficulty in demonstrating this inhibition suggests that the inhibitory fibers in the gastric vagus are more susceptible to reflex than direct stimulation.

SUMMARY

Atropine markedly increased initial and final emptying times in the normal dogs.

The gastric emptying mechanism is very much impaired in the vagotomized dog's stomach.

The effect of atropine in the denervated dogs depended largely upon the time elapsing since the vagotomy. The older the denervation the greater its effect in prolonging initial emptying and widening the area between the normal and atropine curves of gastric emptying. This effect of atropine is considered as a measure of the recovery of gastric tonus after vagotomy.

Atropine did not seem to affect the rate of gastric emptying in the denervated dogs, once the process was started. Consequently, the effect of atropine in delaying gastric evacuation in the normal dogs and as previously reported in human subjects, is attributed to its paralysis of the gastric vagus and not to its action upon the gastric musculature or Auerbach's plexus.

Pilocarpine promoted gastric evacuation in two of the three normal dogs for the first two-thirds of the emptying with a definite delay occurring near the end of evacuation. In the third dog, it greatly hindered emptying.

In the completely denervated dogs, pilocarpine shortened initial and final emptying times to periods much shorter than the normal for normal dogs. Gastric contents were evacuated and pushed through the intestine regardless of their state of digestion. This demonstrates the impotency of the pyloric sphincter in the regulation of gastric evacuation and also the capacity of the intrinsic neuro-muscular mechanism to carry on the coördinated peristalsis required in emptying if given sufficient driving force. In the sympathetically innervated stomachs, pilocarpine failed to cause very short emptying time with meals of coarse consistency. This suggests that the sympathetic nerve endings in the gastric and enteric mucosa may be affected by the physical state of the gastric chyme.

The delay in gastric emptying with pilocarpine in both normal dogs and human subjects was due to the entero-gastric reflex of Thomas. This reflex with its pathway in the vagus nerve acts as a brake to regulate gastric evacuation to the capacity of the intestine. In our studies it functioned remarkably well, even when the stomach was stimulated by pilocarpine and the meal was composed largely of egg yolk and water.

These experimental studies with atropine and pilocarpine demonstrate and emphasize the very important rôle played by the central nervous system through the vagus nerves in the normal mechanism of gastric evacuation.

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CHANGES IN TISSUE METABOLISM IN OESTRUAL, DIOESTRUAL AND SPAYED RATS

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Recent studies by Andersen and Kennedy (1932) and Andersen (1935) have demonstrated an increase in the weight of the adrenal cortex and the pituitary during oestrus in the rat. No change was found in the thyroid. Histological changes were observed in the adrenal cortex and other authors (Charipper and Haterius, 1932; Wolf and Cleveland, 1933) have described histological changes in the pituitary correlated with oestrus. In subsequent papers Andersen and Kennedy (1933) have described an atrophy of the adrenal and thyroid after ovariectomy and a temporary decrease in pituitary weight. The oestrous weight and histological appearance were restored by injection of small doses of amniotin (ovarian hormone) (Andersen, 1934).

It seemed to us probable that these endocrine changes might be accompanied by alterations in the metabolism of tissues. Previous work on the effect of spaying and the oestrous cycle on the metabolism of the whole animal has produced discordant results, probably due in large part to the difficulty of estimating the effect of the voluntary activity of the animals.

Literature. Many efforts have been made to discover the effect of gonadectomy on metabolism. The majority of workers report a decrease in metabolism in the dog, rabbit and rat and all agree that it is decreased in the capon. The remaining observers report no change in metabolism after gonadectomy. There are no reports of an increased metabolism.

Two studies on tissue metabolism after gonadectomy have been reported. Maeda (1930), working on adult rabbits 2 to 3 months after castration, found an increase in the oxygen consumption of the thyroid in both sexes, a decrease in the metabolism of the adrenal and pancreas in the males and an increase in the adrenal oxygen consumption in both sexes. The metabolism of the pancreas in the female and of the spleen and liver in both sexes remains unchanged. The details of the experiments, numbers of animals, etc., are not given. Bungeler (1932) observed the oxygen consumption of the liver in male mice at various intervals after castration and found a slight increase at first and then a return to normal. At operation

the mice weighed 12 to 20 grams. No controls were used. It seems possible that the gradual decrease was an age change. In the light of the following experiments it is possible that the operative procedure, i.e., trauma, anesthesia, tissue repair, may have been responsible for the immediate post-operative increase.

The work on human subjects has produced equally variable results. The literature is given by Korenchewsky (1925).

Oestrus. All of the work on the effect of the female reproductive cycle on basal metabolism has been on rats or man. Szarka (1929) who followed the O_2 consumption of 7 rats found a 25 per cent increase in metabolism in prooestrus and oestrus. Lee (1928), who carefully selected healthy animals with regular cycles, found an average increase of 13 per cent in the heat production during the last 10 hours of dioestrus and the first 6 hours of prooestrus. Both of these experiments are reported in detail and very active animals are eliminated from consideration. Frazer and Wiesner (1929) found a maximum CO_2 output 12–24 hours after oestrus. Hemmingsen (1934) in experiments in which temperature and activity were controlled, the latter by narcosis, found no change in the O_2 consumption. It is evident that the variations in voluntary activity in the oestrous cycle found by Wang (1923), Slonaker (1924), Kinder (1927), and after spaying (Richter, 1933) would be difficult to control in metabolism experiments except by narcosis and may well explain the difference between the findings of Lee and of Szarka and those of Hemmingsen. The experiments in man are summarized by Szarka and by Lee, and are inconclusive.

The cause of the oestrous changes in the rat are considered by Szarka to be a change of the metabolism of the uterus, which he calculates is of the order of 1,000 per cent. That there is a rise in the oxygen consumption of the uterus just before ovulation has since been demonstrated by King (1932) in the sow and by Khayval and Scott in the mouse and rat (1931), although it was not found in the mouse by David (1931). King found this increase to be of the order of 25 per cent, which would lead to an insignificant rise in the oxygen consumption of the whole animal. No other studies on tissue respiration during the cycle have been found.

Experimental. In the following experiments we have been concerned with the in vitro metabolism of liver and kidney of oestrual, dioestrual and spayed rats. The R.Q. and rate of oxygen consumption were studied.

Forty rats were used. They were of the strain which has been inbred in this laboratory for 5 years. The diet and care of the animals is constant and has been described in previous papers (Andersen and Kennedy, 1932). The animals had free access to food and water. The vaginal smears were taken daily for the two weeks before the experiments and only animals with regular cycles of 6 days or less were used. The experiments were of necessity carried out between the hours of 9 and 5 and those animals were

considered in oestrus which had a dioestrous smear at noon of the previous day and nucleated epithelial cells on the morning of the experiment. If a dioestrus smear was followed next morning by cornified cells the rat was left until the next cycle. The dioestrous animals were used 48 or 72 hours after oestrus. The spayed group were ovariectomized on the 60th to 62nd days, and all had open vaginas at that time. All of the animals were between the ages of 96–107 days when killed, which was 6 weeks after operation for the spayed ones. At the beginning of the experiment all animals in which there was evidence of infection were excluded.

METHOD. The rats were killed by a blow on the head. The liver and kidneys were immediately removed and sectioned with a razor blade into slices 0.2 to 0.3 mm. thick. Precautions were taken to use only the cortical tissue of the kidney. To prevent drying during the slicing process, the tissues were moistened with Ringer's solution. After sectioning, the tissues were blotted free of any excess fluid and rapidly weighed on a Roller-Smith torsion balance to 0.2 mgm. . About 100 mgm. of liver and 50 mgm. of kidney were used in each of the vessels for metabolic observations. The O_2 consumption and CO_2 production were determined by the method described by one of us (Victor and Potter, 1935), which is a modification of the Fenn differential volumeter. By this method the O_2 consumption and CO_2 consumption of the same tissue as well as the preformed CO_2 of the tissue were measured in a single respirometer. The tissues were immersed in Ringer solution containing 0.9 per cent NaCl, 0.0236 per cent $CaCl_2$, 0.022 per cent KCl, phosphate buffer pH 7.4 containing 10 mgm. P per 100 cc. and 0.2 per cent glucose. The solutions for absorbing CO_2 and acidification of the tissues were the same as those already described. Pure oxygen was flushed through the respirometer vessels for about 5 minutes. About ½ hour elapsed between the death of the animal and the first metabolic observations. The water bath temperature was 37.5° C. ± 0.005. The respirometers were shaken through an arc of about 5 cm. at the rate of 180–200 times per minute. Observations lasted 1 to 2 hours in the case of liver and ½ to 1 hour with kidney tissue. No change in rate of oxygen consumption of either the liver or kidney was noted in this time.

The pituitary, thyroid and adrenal glands were dissected out and weighed. The weights of the gland confirmed our previous findings. The lungs and middle ears of each rat were examined for evidences of infection and the infected rats are considered separately. In no case was the infection sufficiently severe to affect the body weight.

Data. The body weight at autopsy varied from 154 to 196 grams for the normal animals and from 201 to 236 grams for the uninfected spayed animals. The mean body weights are as follows: uninfected animals: oestrous 175±2.7, dioestrous 178±2, spayed, 218±3.4 grams; infected spayed animals: 219±3.

TABLE 1

Respiratory rates and quotients of rat liver and kidney—oestrus, dioestrus and spayed

O_2 CONSUMPTION	R.Q.	O_2 CONSUMPTION	R.Q.
Liver	Oestrus (age 98–101 days)	Kidney	
Cmm./gm./min.		*Cmm./gm./min.*	
19.3	0.77	46.4	1.00
17.3	0.83	51.5	1.02
19.5	0.85	50.3	0.90
19.4	0.85	57.8	0.99
16.4	0.78	50.0	0.97
16.4	0.77	45.3	0.88
16.1	0.70	44.7	1.00
17.0	0.81	46.5	0.93
24.2*	1.00	47.5	1.00
22.7†	1.00	47.6	0.87
16.0‡	0.83	57.3	1.00
18.3‡	0.86	46.0	0.98
	Dioestrus (age 96–107 days)		
17.0	0.83	49.6	0.94
13.4	0.80	41.8	0.92
15.1	0.78	44.8	1.01
16.9	0.93	47.2	1.00
14.4	0.89	47.6	0.98
16.2	0.95	56.6	0.98
14.4	0.83	43.8	0.98
14.3	0.80	48.8	0.81
14.6	0.70	46.6	0.97
12.6	0.70	47.0	0.98
	Spayed (age 101–103 days)		
14.7	0.85	52.6	1.02
12.8	0.81	52.6	0.80
11.5	0.75	55.2	0.98
11.8	0.78	40.5	0.96
14.4		44.0	0.88
12.0	0.70	40.9	0.95
14.8	0.84	48.0	1.00
14.7	0.76	55.8	0.83
13.3	0.70	51.7	0.88
16.4‡	0.96	58.4	1.00
17.8‡	0.97	47.9	1.00
16.1‡	0.84	49.9	1.03
16.2‡	0.86	52.5	1.00
13.8‡	0.86	43.0	0.93
12.2‡	0.82		
17.1‡	0.87	48.0	0.93
15.7‡	0.78	53.8	0.93
19.4‡	0.76	54.6	0.88

* 2 ears pus, pneumonia.

† 2 ears pus.

‡ 1 ear infected—scar, granulation tissue.

RESULTS. In table 1 are recorded the data of observations, the age of the animals, their reproductive state and the incidence of infection among them. Table 2 gives mean values, standard deviations and probable errors of the means. Table 3 shows the differences between the means for the tissues from rats that were spayed or in oestrus or dioestrus, as well as the

TABLE 2

Summary. Respiratory rates and quotients of rat liver and kidney—oestrus, dioestrus and spayed

STAGE	NUMBER OF RATS	LIVER				KIDNEY			
		O_2 consumption		R.Q.		O_2 consumption		R.Q.	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
		cmm./gm./min.	*cmm./gm./min*			*cmm./gm./min.*	*cmm./gm./min.*		
Oestrus.....	8	17.7±0.24	1.0	0.795±0.014	0.06	49.1±0.83	3.5	0.961±0.012	0.05
Oestrus-infected....	4	20.3±1.1	3.3	0.922±0.028	0.08	49.7±1.4	4.2	0.962±0.021	0.06
Dioestrus.....	10	14.9±0.25	1.2	0.821±0.017	0.08	47.4±0.75	3.5	0.957±0.012	0.06
Spayed.....	9	13.3±0.36	1.7	0.774±0.012	0.05	49.6±1.25	5.6	0.923±0.016	0.07
Spayed-infected....	9	16.1±0.45	2.0	0.858±0.015	0.07	51.0±1.0	4.5	0.962±0.011	0.05

TABLE 3

Metabolic differences between oestrual, dioestrual, spayed and infected rats

COMPARISON OF	LIVER				KIDNEY			
	O_2 consumption		R.Q.		O_2 consumption		R.Q.	
	Diff.	Diff. / P.E. Diff.	Diff.	Diff / P.E. Diff.	Diff.	Diff. / P.E. Diff.	Diff.	Diff. / P.E. Diff.
	cmm./gm./min.	*cmm./gm./min.*			*cmm./gm./min.*	*cmm./gm./min.*		
Oestrus-dioestrus.....	*2.8*±0.35	8.0	*0.026*±0.022	1.2	1.7±1.12	1.5	0.004±0.017	0.2
Dioestrus-spayed.....	*1.6*±0.44	3.7	*0.047*±0.021	2.2	2.2±1.46	1.5	0.034±0.02	1.7
Oestrus-spayed.....	*4.4*±0.43	10.2	*0.021*±0.019	1.1	0.5±1.5	0.3	0.038±0.02	1.9
Oestrus-infected.....	2.6±1.13	2.3	*0.127*±0.031	4.1	0.6±1.62	0.4	0.001±0.024	0
Spayed-infected.....	*2.8*±0.57	4.9	*0.084*±0.019	4.4	1.4±1.61	0.9	0.039±0.019	2.0

The italicized values are statistically significant.

differences between normal and infected rats; probable errors of the differences are given as well, with the ratio of the difference to its probable error. When this ratio is over 3 (statistically significant), it is italicized. Figure 1 is a scatter chart of individual observations in oestrual, dioestrual and spayed animals.

There were significant differences in the rates of oxygen consumption of

the livers of oestrual, dioestrual and spayed rats. The former showed the highest rates and the latter the lowest with the dioestrual intermediate. The kidney, on the other hand, showed no changes in respiratory rates or quotients. The R.Q. of the livers were not significantly altered in the various stages. The respiratory quotients of kidney tissue in this series were higher than those reported by other observers. The presence of glucose in the Ringer's solution probably accounts for the difference.

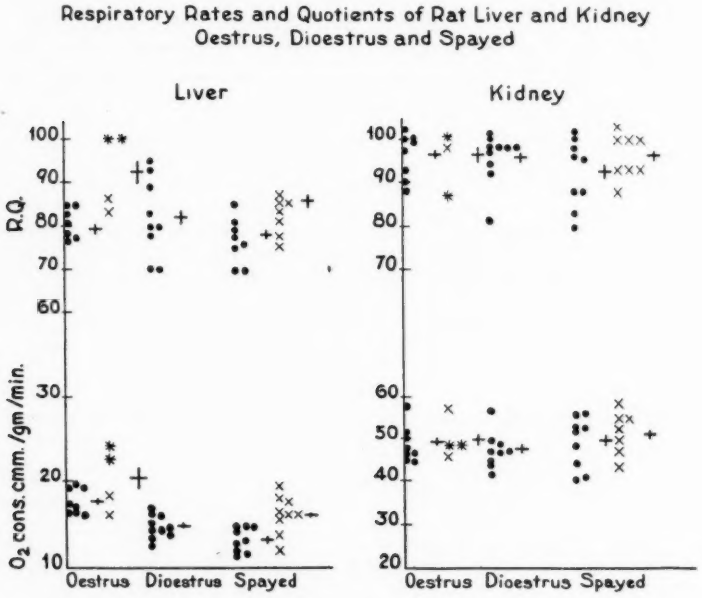

Fig. 1. Scatter chart of individual respiratory rates and quotients of liver and kidney in oestrual, dioestrual and spayed rats.

● no infection; * pyogenic infection; × slight infection; − mean; | 2 × probable error of mean.

Richardson, Loebel and Schorr (1926) found the R.Q. of kidney in glucose to be 0.888. They studied the metabolism over a period of about 2 hours. In our experiments the R.Q. of kidney was determined over a period of ½ to 1 hour. It is possible that the R.Q. of kidney decreases with time. According to Fenn (1932) this occurs in frog skeletal muscle. It has been shown that the R.Q. of kidney, testis, spleen and brain is increased by glucose (Dickens and Greville, 1933). According to Dickens and Simer (1931) the R.Q. of liver in Ringer phosphate medium was 0.79, which agrees fairly

well with the mean values of 0.795, 0.821 and 0.774 obtained in this series for the oestrual, dioestrual and spayed rats respectively.

There were metabolic differences between the livers of infected and normal rats. The kidneys, however, showed no changes. The respiratory rates and quotients of the livers of the infected rats were elevated. This was observed in both the oestrus and spayed series. No infected rats were studied in the dioestrus series. It is not known whether the temperature of the animals with increased liver metabolism was elevated. The elevated respiratory rates of the liver do not appear to be secondary to elevated temperature change since the metabolism was determined at a uniform temperature, namely, 37.5°C. Not all of the animals with infections showed metabolic changes. These differences in metabolic response may have been due to differences in the type of organism responsible for the infection. No attempt was made to determine the nature of the infection. It has been found that the basal metabolic rates of man vary in different diseases and also in the same disease varying with the body temperature (DuBois, 1927). The in vitro observations on the infected rats indicate that the metabolism of liver tissue may be altered independently of the environmental temperature. The increased R.Q. of the livers of these infected rats indicates an accelerated utilization of glucose by these tissues. The rôle of the liver in intoxication and infection and the effect of carbohydrates in mitigating the toxic effects has been well established by Opie and Alford (1914). The increased utilization of glucose by livers of infected rats suggests an increased demand for this substance by this tissue under these conditions. Whether these tissues show a high R.Q. in the fasting state and in the absence of glucose has not been determined.

Bungeler reported that the oxygen consumption of mouse livers first increased and then returned to normal after castration. The operative procedure is associated with tissue trauma, intoxication due to anesthesia, absorption of disintegrated tissue production, and greater possibilities of respiratory infections. The post-operative rise in liver metabolism may have been a result of the operation and not directly due to castration. In our series the greatest change in liver metabolism occurred in those with the most severe infection (table 1).

Discussion. The metabolic changes that occur in the tissues of oestrual, dioestrual and spayed rats are not generalized. This is indicated by the change that occurs in the liver but not in the kidney. According to Davis and Hastings (1934) the O_2 consumption of a sleeping 4 month old rat, approximately the age of rats of these experiments, is 21.2 cc./gm./24 hrs. or 14.7 cmm./gm./min. If one assumes that the metabolic rate of tissues in vitro is the same as that in vivo, the following correlations may be permitted. A 15 per cent change in liver metabolism, the difference

between oestrus and dioestrus, would produce only a 0.3 per cent change in total metabolism, assuming that the metabolism of the other tissues was unaffected. This change could, of course, hardly be detected in measurements of the total metabolism. According to Hemmingsen, who has made a larger number of observations of total metabolism in oestrual and dioestrual rats (over 100 in each series) than any other author, the metabolic difference in these states was about 1 per cent. This difference in total metabolism may be significant. However, metabolic changes may occur in other tissues. An increase has been found in the uterus (27), but this is correlated with an increased frequency of contractions.

The cause of these metabolic differences in oestrus are not apparent. The changes in the weight of the pituitary gland during the oestrous cycle indicate that this organ may play a rôle through its thyrotropic function. The decreased oxygen consumption of the liver in spayed animals may be due to the thyroid atrophy and probable hypofunction that occurs after spaying. Under such conditions a differential metabolic effect of thyroxin should be demonstrable between liver and kidney.

McEachern (1935) has recently reported a difference in the metabolic response of kidney as compared with other tissues following thyroxin injections. In the same animals the respiratory rate of liver and diaphragm was increased 75 per cent in contrast to a 46 per cent increase in kidney metabolism. It is possible that smaller changes in thyroid activity may be associated with no changes in renal respiration, but definite changes in the metabolism of other tissues.

The quantitative and qualitative changes observed in the metabolism of livers from infected animals indicate that precautions must be taken to eliminate this factor in experiments of this type.

SUMMARY AND CONCLUSIONS

1. The respiratory rates and quotients of liver and kidney of oestrual, dioestrual and spayed rats were studied in vitro.

2. The respiratory rates of the liver changed under these conditions. The rate of oxygen consumption of the liver was highest in oestrual and lowest in spayed rats. The livers of dioestrual rats had an intermediate metabolic rate. The R.Q. was unaltered.

3. The metabolism of the kidney did not vary in any of these states.

4. Infection in the rat increased the respiratory rates and quotients of the liver, but did not affect the kidney.

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THE EFFECTS OF OESTRUS AND SPAYING ON PITUITARY METABOLISM

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The anterior lobe of the pituitary gland undergoes cytologic (4, 13) and weight changes (1, 3) during the oestrous cycle and after spaying. Smith and Engle (8) and Wolfe (12) have shown that there are alterations in the amount of gonadotropic substance in the anterior lobe in these states. The object of the present studies has been to investigate the metabolism of the anterior lobe tissue in vitro. The rates of oxygen consumption and aerobic and anaerobic glycolysis were measured in prooestrual, oestrual, dioestrual and spayed rats.

METHOD. The rats were of the same age as those used in previous experiments (10) being from 97 to 108 days old. Daily vaginal smears were taken to determine the stage of the oestrous cycle. Oestrus was defined as the period at which an abundance of cornified cells was obtained. Dioestrus was 48 to 72 hours after this time. In the case of prooestrus observations, smears were taken at 8 hour intervals and prooestrus was defined as the time when the first smear containing nucleated epithelial cells was obtained. All the spayed rats were operated upon 41 to 43 days before the metabolic determinations.

The breed, diet and care of the animals were similar to that previously described (2). Complete autopsies were performed on all the rats. Data from infected animals are not included, although no correlation was found between the pituitary metabolism and the presence of infection. The most common spontaneous infection is that of the middle ear and the ears were inspected in each case. Previous studies on liver and kidney metabolism have demonstrated that infection increases the respiratory rate and quotient of the liver in the presence of glucose (10).

The pituitary glands were removed after killing the rats with a blow on the head. The anterior lobe was separated from the posterior one and cut into slices about 0.2 to 0.3 mm. thick. The tissue was immersed in Ringer's solution during the sectioning to prevent evaporation. In each case only a portion of the anterior lobe was used, about 4 mgm. being sufficient for a complete metabolic determination. Approximately 1 mgm.

of tissue was placed in each of four respirometers. The rates of oxygen consumption and aerobic and anaerobic glycolysis were measured in respirometers of the type described by one of us (9). After blotting to remove excess fluid the tissue was rapidly weighed to 0.02 mgm. on a blotting-paper pan of a Roller-Smith torsion balance. To prevent evaporation during the period of weighing, which lasted about 15 seconds, a dish containing Ringer solution was placed in the chamber that encircled the weighing pan. The oxygen consumption was measured in Ringer solution consisting of 0.9 per cent NaCl, 0.0236 $CaCl_2$, and 0.022 KCl with phosphate buffer pH 7.4 containing 10 mgm. P per 100 cc. The CO_2 was absorbed by 0.2 N NaOH on filter paper placed in the capillary. The aerobic and anaerobic glycolysis were measured in Ringer-bicarbonate-glucose solution containing $2.5 \times 10^{-2}M$. $NaHCO_3$ and 0.2 per cent glucose in equilibrium with 5 per cent CO_2 in 95 per cent O_2 and N_2 respectively. A control respirometer containing glucose-free Ringer-bicarbonate solution in equilibrium with 5 per cent CO_2-95 per cent O_2 was used for determining the respiratory CO_2. The N_2-CO_2 mixture was flushed over copper filings at 700°C. to remove any oxygen. The respirometers were shaken through an arc of about 5 cm., 140 times a minute. The water bath temperature was 37.5°C. $\pm$ 0.005.

Results. In table 1 are recorded data of the individual observations of oxygen consumption and aerobic and anaerobic glycolysis. Table 2 gives the mean values, standard deviations and the probable errors of the means. Table 3 shows the differences of the means of each stage of the oestrous cycle and the differences between the spayed and the other stages; the probable errors of the differences with the ratio of the difference to the probable error are also included. When this ratio is 3 or over, it is italicized. Figure 1 is a scatter chart of the individual observations. Figure 2 is a graphic representation of the means of the rates of oxygen consumption and their probable error. It also gives the mean values of the weight in a comparable series reported by one of us (1, 3) as well as the total respiratory metabolism of the pituitary obtained by multiplying the gland weight by the metabolic rate.

There were differences in the rates of O_2 consumption of the anterior pituitary of prooestrual, oestrual and dioestrual rats. No significant differences were noted between the rates of O_2 consumption of dioestrual and spayed rats. Comparisons of pituitaries showed that those of rats in oestrus had lower respiratory rates than those in prooestrus. When the rates of O_2 consumption of pituitaries of prooestrual were compared with dioestrual or spayed rats, they were found to be higher than either.

So far as the rate of aerobic glycolysis is concerned, there were statistically significant differences only between the anterior pituitaries of dioestrual and spayed rats. However, it is unknown whether glucose affects

TABLE 1

Metabolic rates of rat pituitary—prooestrus, oestrus, dioestrus and spayed

REPRODUCTIVE STATES	O_2 CONSUMPTION	AEROBIC GLYCOLYSIS—CO_2 EQUIVALENT TO ACID PRODUCTION	ANAEROBIC GLYCOLYSIS—CO_2 EQUIVALENT TO ACID PRODUCTION
	c.mm./gm./min.	*c.mm./gm./min.*	*c.mm./gm./min.*
Prooestrus	16.5	3.3	26.8
	16.1	2.4	38.1
		2.2	22.4
	16.0	2.8	21.3
	16.8	4.8	28.4
	14.4	5.7	26.5
	14.7	7.9	24.6
	13.7	3.3	19.3
	16.2	4.4	18.6
Oestrus	13.5	8.4	20.9
	10.0	3.6	26.2
	15.5	8.2	33.7
	17.5	1.8	17.3
	12.9	0	14.1
	10.6	2.1	30.4
	13.3	2.8	26.8
	15.3	6.8	18.8
	11.2	4.1	21.3
Dioestrus	13.4	0.8	15.4
	14.9	3.5	18.9
	10.7		14.2
	12.6		16.0
	8.7	2.4	18.7
	12.6		25.5
	7.8	2.3	21.0
	9.8	0.9	24.4
	10.1	4.3	22.3
	11.1	5.1	16.0
	12.2	1.8	16.3
Spayed	10.7	0.8	16.2
	12.2	4.8	20.6
	9.1	8.5	21.9
	12.5	3.4	20.0
	10.2	4.1	17.4
	10.8	3.7	20.6
	8.0	3.7	16.8
	12.6	5.1	20.2
	11.3	4.3	15.8
	10.5	4.3	23.6
	10.5	5.6	19.4

either the R.Q. or the rate of O_2 consumption of pituitary tissue. The small amount of glycolysis measured as CO_2 in these observations may be due to the effect of glucose on the R.Q. or rate of O_2 consumption and not at all to glycolysis. The sources of error in glycolysis measurements by this method have been previously indicated (5, 11).

The rate of anaerobic glycolysis of the pituitaries was significantly higher in the prooestrual than in either the dioestrual or spayed rats. Although

TABLE 2

Summary. Metabolic rates of rat pituitary—prooestrus, oestrus, dioestrus and spayed

	O_2 CONSUMPTION		AEROBIC GLYCOLYSIS —CO_2 EQUIVALENT TO ACID PRODUCTION		ANAEROBIC GLYCOLYSIS—CO_2 EQUIVALENT TO ACID PRODUCTION	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
	c.mm./gm./min.	*c.mm./gm./min.*	*c.mm./gm./min.*	*c.mm./gm./min.*	*c.mm./gm./min.*	*c.mm./gm./min.*
Prooestrus	15.5±0.38	1.6	4.1±0.40	1.7	25.1±1.32	5.6
Oestrus	13.3±0.53	2.4	4.2±0.62	2.8	23.3±1.34	6.0
Dioestrus	11.3±0.36	1.8	2.6±0.35	1.4	18.9±0.91	4.5
Spayed	10.8±0.20	1.0	4.4±0.29	1.4	19.3±0.50	2.5

TABLE 3

Differences in pituitary metabolism at various stages of the oestrous cycle

COMPARISONS OF	O_2 CONSUMPTION		AEROBIC GLYCOLYSIS—CO_2 EQUIVALENT TO ACID PRODUCTION		ANAEROBIC GLYCOLYSIS—CO_2 EQUIVALENT TO ACID PRODUCTION	
	Diff.	$\frac{\text{Diff}}{\text{P.E. Diff.}}$	Diff.	$\frac{\text{Diff.}}{\text{P.E. Diff.}}$	Diff.	$\frac{\text{Diff.}}{\text{P.E. Diff.}}$
	c.mm./gm./min.	*c.mm./gm./min.*	*c.mm./gm./min.*	*c.mm./gm./min.*	*c.mm./gm./min.*	*c.mm./gm./min.*
Oestrus-dioestrus	*2.0*±0.64	3.1	1.6±0.71	2.3	4.4±1.62	2.7
Oestrus-spayed	*2.5*±0.57	4.4	0.2±0.68	0.3	4.0±1.43	2.8
Oestrus-prooestrus	*2.5*±0.65	3.8	0.1±0.74	0.1	1.8±1.88	1.0
Dioestrus-spayed	0.5±0.41	1.2	*1.8*±0.46	3.9	0.6±1.04	0.6
Dioestrus-prooestrus	*4.2*±0.52	8.1	1.5±0.53	2.8	*6.2*±1.60	3.9
Spayed-prooestrus	*4.7*±0.43	11.0	0.3±0.49	0.6	*5.8*±1.41	4.1

Significant differences are italicized.

the mean value of anaerobic glycolysis for oestrus was higher than dioestrus and spayed animals, it was not statistically significant.

DISCUSSION. The only observations on pituitary metabolism we have been able to find are those of Fujita (6) who studied the QO_2, $Q_{CO_2}^{O_2}$, and $Q_{CO_2}^{N_2}$ of pituitaries pooled from four rats. The values he obtained for

these were 8, 1, and 13 respectively. The amount of the gonad stimulating hormone in the pituitary has been shown to be greater in dioestrus than in oestrus (Smith and Engle and Wolfe), and greatest in spayed animals (Smith and Engle). The weight of the pituitary gland has been found to be greater in oestrus than in dioestrus (1). Furthermore, the weight of the gland in rats 6 weeks after spaying is about the same as in dioestrus (3). In other words, so far as the oestrual cycle is concerned, the weight of the gland is inversely proportional to its gonad stimulating content. On the other

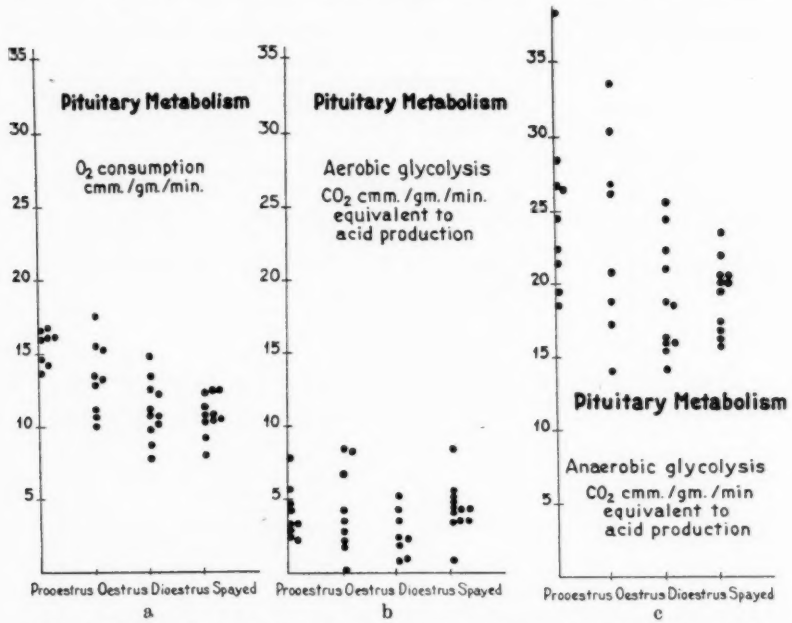

Fig. 1. Scatter chart of rates of oxygen consumption and aerobic and anaerobic glycolysis of pituitaries. Rats at prooestrus, oestrus, dioestrus and 6 weeks after spaying. There are differences in rates of O_2 consumption and anaerobic glycolysis between prooestrus, oestrus and dioestrus but not between dioestrus and spayed.

hand, the large gland in spayed animals contains more of the hormone than that in normal rats. The metabolic activity of the pituitary was greatest in prooestrus and least in dioestrus and after spaying. In oestrus the metabolic rate was intermediate. The metabolic rate of the gland was at its peak when the weight of the gland had begun to increase. If the relative weight of the gland (1, 3) (fig. 2) is multiplied by the metabolic rate, the total metabolism of the gland is found to be highest and about the same in prooestrus and oestrus, and lowest in dioestrus, being 0.914,

0.878, and 0.606 cmm. O_2 per kgm. body weight per minute respectively. The weight and total metabolism of the gland in spayed rats is about the same as in dioestrus, the former being 0.614 cmm. O_2 per kgm. body weight per minute. The difference in total pituitary metabolism in prooestrus or oestrus and dioestrus was about 50 per cent. The total metabolism then was found to be highest in oestrus and lowest in dioestrus and, therefore, inversely proportional to the gonad stimulating content.

Several possible explanations for the inverse relationship of the metabolism to the gonad stimulating content may be mentioned.

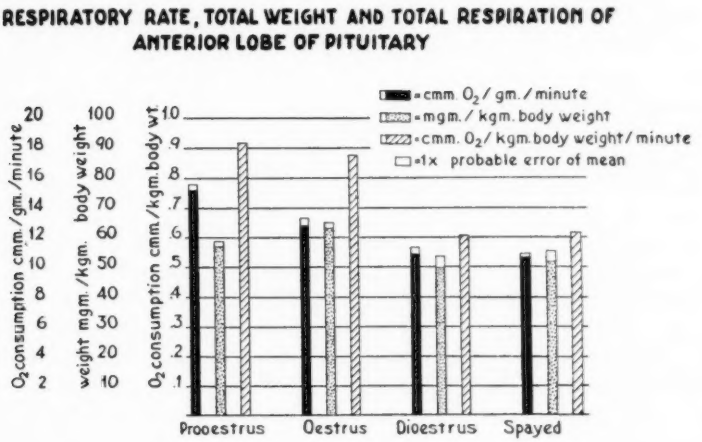

Fig. 2. Mean rates of O_2 consumption of pituitaries of rats at prooestrus, oestrus, dioestrus and 6 weeks after spaying. The rates per gram per minute and per kilogram body weight per minute are represented as well as the weight in milligrams per kilogram body weight. Although the respiratory rate of the pituitary is higher at prooestrus than at oestrus, the total respiration of the pituitary is the same in both these states because of the larger pituitary at oestrus.

The gonad stimulating hormone may be elaborated at its greatest rate and discharged more rapidly in prooestrus and oestrus. The energy for its formation may be derived from the accelerated respiratory metabolism. During dioestrus and in spayed rats its formation and elimination may be partially suppressed with subsequent accumulation of the gonad stimulating hormone. A parallel to this is found in the inferior potency and iodine content of actively growing thyroid as seen in exophthalmic goiter, as compared with resting thyroid (7). However, the respiratory rates of actively growing and resting thyroid cells have not been compared.

The total energy production of the tissue may be unaltered throughout the oestrous cycle. In this case the energy that may be derived from the

elaboration of the pituitary hormones may be independent of aerobic processes and replace the respiratory energy. This would imply that the elaboration of the hormones occurred at its greatest rate during dioestrus and after spaying and that the resting state was during prooestrus and oestrus. Such an hypothesis might be compatible with the gonad stimulating content of the pituitary. However, the other endocrine changes militate against this. It appears more probable that the high gonad-stimulating content of the gland in dioestrual and spayed rats indicates greater storage rather than greater production of the hormone.

The metabolic changes of the pituitary may be similar to those occurring in the liver in various reproductive states. That it is not the same is seen from the fact that the metabolic rates of the pituitary in dioestrus and after spaying are the same. In liver, on the other hand, the dioestrous rate is considerably higher than the spayed. Although the pituitary and liver metabolism are correlated with changes in the reproductive activity of the female rat, these changes are not part of a generalized metabolic alteration. This is seen in the fact that kidney metabolism does not vary under the same conditions (10).

It has been shown that metabolic differences exist in the livers of oestrual, dioestrual and spayed rats, being highest in the former and lowest in the latter. These changes in liver metabolism may be secondary to changes in thyroid activity since the lowest metabolism occurs in spayed rats, when thyroid atrophy is present. Since the pituitary influences thyroid activity it is possible that the liver changes may be secondary to the formation of thyrotropic hormone by the pituitary in prooestrus. The weight of the adrenal glands has been found to be higher in oestrus than in dioestrus. These changes may likewise be secondary to pituitary influence, either directly or via the thyroid. If any or all of these changes occurring in the oestrous cycle are initiated by the pituitary gland, then a 50 per cent increase of the oxidative energy of the pituitary is necessary for the elaboration of the hormones.

It may be permissible to indicate that a study of the metabolism of the pituitary is the only method for measuring pituitary activity that does not require a third variable. In other words, the measurement of pituitary activity depends only on the host and the pituitary cells and not upon any recipient of these cells as is necessary for the demonstration of gonadotropic, thyrotropic, adrenotropic or any other hormones that may be present. Of course, the respiratory response is probably not specific for any of these pituitary functions, but the resultant of several or all of them.

SUMMARY AND CONCLUSIONS

1. The rates of oxygen consumption and aerobic and anaerobic glycolysis of the anterior lobe of the rat pituitary were observed in vitro. Prooestrual, oestrual, dioestrual, and spayed rats were used.

2. Significant differences in respiratory rates were observed. The respiratory rates were highest in prooestrus, lowest in dioestrus and intermediate in oestrus. The pituitaries of the spayed rats had the same respiratory rates as those of dioestrual rats.

3. The pituitaries had significantly higher rates of anaerobic glycolysis in prooestrual than in spayed or dioestrual rats. Those in oestrus also had higher rates than the latter two series.

4. The total pituitary metabolism was about the same in prooestrual and oestrual rats and higher than in spayed or dioestrual rats. That of spayed rats was the same as that of dioestrual rats.

5. It cannot be concluded from these experiments that significant differences in the rates of aerobic glycolysis exist in these reproductive states.

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AGE AND OTHER FACTORS IN MOTOR RECOVERY FROM PRECENTRAL LESIONS IN MONKEYS[1]

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In man and in subhuman primates the paresis produced by lesions of the motor cortex is variable in respect of rate and of extent of ultimate recovery of motor power. Clinically various factors influencing recovery are recognized, such as the size and nature of the lesion and its location in the cerebral hemispheres, but the extent of recovery is generally unpredictable. In an experimental study these factors can be analyzed more precisely, and it is the purpose of this paper to present the results of such a study of the problem carried out on monkeys from which the various parts of the excitable motor regions of the cortex were extirpated. The regions removed were the motor and premotor areas, i.e., area 4 and area 6 a (upper part) of Vogt's modification of Brodmann's architectonic map of the cercopitheque brain (1, fig. 1). Following extirpation observations were made on the rate and degree of ultimate recovery of motor power. The effects produced by such lesions were found to differ strikingly in the infant monkeys from those seen in adult animals.

METHOD. During the past five years investigations of the functions of the frontal lobe of subhuman primates have been carried out at the Yale School of Medicine under the direction of Prof. J. F. Fulton (2, 3, 4, 5). The observations reported in this paper have therefore been made incidentally on a large number of animals operated upon by Doctor Fulton for other purposes, and, in addition, on a smaller number used specifically for this research. Motor pareses of one or more extremities were produced in monkeys of various species, in gibbons and in chimpanzees by total and subtotal ablations of the excitable regions of the frontal lobe. In 13 monkeys the motor and premotor regions were completely removed from *both* hemispheres (5).

Recovery of function was measured in several ways. The changes in activity of the deep reflexes of the lower extremities were noted, and, in the apes, the appearance of the pathological reflexes of the foot, the Babinski, Rossolimo, Chaddock and Oppenheim; the degree of resistance to passive manipulation (*i.e.*, spasticity and flaccidity) and, with this, the presence or absence of reflex grasping, its time of appearance and disappearance, and its intensity were also observed. In addition the behaviour of the animals on the return of voluntary power was carefully studied;

[1] This investigation was assisted by a grant from the Research Funds of the Yale University School of Medicine.

the phenomena ordinarily noted were: 1, movement at a given joint, shoulder, elbow, wrist, fingers, hip, knee, ankle and toes; 2, ability to use the extremity for support; 3, ability to use the extremity for walking and whether the dorsum of the foot or hand bore weight rather than the sole as in the normal animal; 4, ability to use the extremity in climbing; 5, prehension, *i.e.*, use of the toes and fingers in picking up large and very small objects; 6, use of the operated extremity in voluntary motor performance as freely, as easily, and as often as the contralateral extremity.

In the 13 animals with bilateral extirpations, histological examination of the excised areas was made by Nissl's method. The surrounding cortical tissue was also preserved at autopsy and regions adjacent to the lesion were similarly examined by Nissl technique. Special attention was paid to the presence or absence of the large pyramidal cells of Betz. In the series with bilateral extirpations the premotor area and all the motor area containing Betz cells was ultimately extirpated. In each instance in which the experiment was terminated within six weeks of the final lesions, the degeneration in cord and brain-stem were studied by Marchi's method.

EXPERIMENTS. A. *Extirpation of motor and premotor areas in adult and subadult monkeys.* 1. *Influence of the age of the animal.* As the number of observations gradually increased it became evident that the age of the animals studied was an important factor in the ultimate degree of motor deficit. Thus adult monkeys from which motor and premotor areas had been removed from one side showed a greater degree of paresis immediately after operation and slower recovery of power than did adolescent or very young animals after the same procedure. The degree of ultimate recovery was also greater in the younger than in the older animals.

2. *Influence of size of lesion (unilateral).* The size of the lesion, as is well known in man, also affects the rate of recovery; being always more rapid after a small lesion than after a large one. Thus, if the entire motor area, *i.e.*, the representation of foot, leg and trunk, as well as arm, was extirpated at one time, the rate of recovery of function *in the hand and arm* was slower than after ablation of the hand and arm area alone. Similarly, extirpation of either the motor or premotor area alone was followed by more rapid and more complete recovery than that following the simultaneous extirpation of both these regions. The influence of size of lesion applied only within the motor and premotor areas. Extirpation of the frontal regions anterior to areas 4 and 6, was performed without increasing the motor deficit. In three cases one entire hemisphere was removed. The ultimate motor deficit in such animals was no greater than that following ablation of motor and premotor areas alone.

3. *Influence of the ipsilateral hemisphere.* The motor and premotor areas in the ipsilateral hemisphere also influence recovery of power. Thus, if an animal from which the excitable motor area of one hemisphere has been removed, is allowed to survive several weeks or months, the motor deficit in the contralateral extremities gradually diminishes, until a permanent level is reached, at which time the animal shows a certain amount of power and

skill on the affected side. If then the corresponding motor area is removed from the second hemisphere, in addition to the deficit which appears in the side contralateral to this second operation, there is marked increase in the deficit of the ipsilateral side as well (12).

4. *Influence of interval between ablations from opposite hemispheres.* Bieber and Fulton (5) found that when the motor and premotor areas were removed bilaterally from adult monkeys the animals never recovered voluntary power. Such animals exhibited only certain involuntary movements and the rhythmic progressive movements of the "thalamic" monkey. They were entirely unable to feed themselves and remained lying on one side in characteristic postures (fig. 1) throughout the remainder of their

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Macaque (premotor 15), after bilateral extirpation of excitable motor areas, showing characteristic posture and inability to move.

Fig. 2. Macaque (premotor 28) a normal infant macaque at 10 days, showing reflex grasping.

Fig. 3. Macaque (premotor 28) at the age of two years, after bilateral extirpation of motor and premotor areas.

lives. The animals studied by Bieber and Fulton were adolescent or young adult macaques (aged 2 to 4 years). They were operated on in two stages, the motor and premotor areas of one side were removed at one operation, and, in the majority of animals, the corresponding area was removed from the second side within two to three weeks.

In the present series of experiments it was found that a slight degree of voluntary power might return, even in adult animals, if the interval between the operations on the two hemispheres were greater than three to four weeks (see table 1). In the majority of animals motor and premotor areas were removed simultaneously from one side at a single operation (the left being the side of primary operation in all cases). The interval between

operations in the various experiments can be seen in the table. Seven instances occurred of recovery of some degree of voluntary power on the right side, and *recovery occurred only in the animals in which a time interval of more than four weeks had elapsed between operations.* Performance three weeks after the extirpation of the motor and premotor area from one hemisphere was usually maximal for the operated extremities, the gradual improvement in power having ceased. Except in two instances the recovery was seen on the right side only, *i.e.*, on the side in which, because of a long interval between operations there had been a maximal recovery of voluntary power. Two cases showed some voluntary movement on the left or more recently operated side, as well as on the right.

TABLE 1

Showing ultimate paresis of voluntary movement in bilateral motor and premotor preparations

The series is listed in order of the time interval between operations. Those animals with short time interval show complete paresis and those with long time interval show incomplete paresis.

	SERIES NUMBER	INTERVAL BETWEEN OPERATIONS	FINAL DEGREE OF PARESIS	
			Left extremities	Right extremities
Young or mature animals:				
Premotor	22	4 days	Complete	Complete
Premotor	16	11 days	Complete	Complete
Premotor	15	17 days	Complete	Complete
Area 6	1	35 days	Complete	Incomplete
Premotor	8	6 weeks	Complete	Incomplete
Premotor	20	6 weeks	Incomplete	Incomplete
Premotor	30	2 months	Complete	Incomplete
Premotor	11	2 months	Complete	Complete
Premotor	39	2 months	Complete	Incomplete
Premotor	24	3 months	Complete	Incomplete
Premotor	4	3 months	Incomplete	Incomplete

Voluntary power was never more than minimal in these older animals. It consisted solely in a response of an extremity in performing a purposeful movement. Some animals were able to propel a bit of food along the floor towards their mouths with their hands, others could awkwardly grasp the food and transfer it to their mouths. Most were able to right themselves, a few could stand, and one (premotor 4) in which a period of 15 weeks had elapsed between operations, was able to progress in an awkward fashion. None of these animals was in any way capable of caring for itself. Those in which the motor representation for the face area had been left could chew and swallow food which was placed in their mouths.

B. *Extirpation of motor and premotor areas in infant monkeys.* Because

of the extreme character of the deficit in older animals, the effect of similar lesions in very young animals was next investigated. The youngest animals previously studied were at least two years of age. Through the courtesy of Dr. Gertrude van Wagenen of the Yale Department of Obstetrics and Gynecology several new-born infant macaques (*Macaca mulatta* and *Macaca irus*) were made available. Infant monkeys at this age show many of the reactions seen in older animals after ablation of the excitable areas. Motor progression is awkward and forced grasping (fig. 2) is very pronounced (8). They exhibit changes in intensity of the reflex grasp on changing position, similar to those seen in the older animals after bilateral extirpation of the motor areas (5). Thus with the animal in the lateral position, the undermost extremities are extended, the uppermost are flexed and exhibit reflex grasping. Reversal of the position of the animal to the opposite side results in reversal of the postural pattern.

1. *Unilateral lesions.* a. *Motor and premotor.* From such an animal (premotor 28) at the age of 10 days the left motor and premotor areas were removed. The immediate recovery after the operation was surprising. Within 24 hours the animal walked about, using all four extremities, with only a slight lag in those of the right side. In purposeful movement, as grasping or picking up an object, the right fingers and toes were used less frequently and a trifle more awkwardly than the left, but even this disability disappeared within ten days. Forced grasping, after the first day when the right hand and foot showed weakness, was at all times equal on the two sides. It disappeared gradually during the second month of life and simultaneously on the two sides of the body. This animal then developed at a normal rate for a healthy infant and showed no motor deficit.

b. *Entire hemisphere.* A second infant (premotor 42) was operated on at the age of 40 days. From it the entire left hemisphere was removed. On recovery from anesthesia, during the first day after the operation, it showed the characteristic deficit of an adult animal after extirpation of a hemisphere: hemianopsis, loss of sensibility and motor paresis were present on the contralateral side. An adult during several weeks after hemispherectomy gradually recovers the use of the operated extremities for motor progression, but gross incoördination is present and ability to perform fine movements is permanently lost.

In this infant, recovery after twenty-four hours was as great as after several weeks in the adult. When returned to its mother at the end of forty-eight hours it clung with all four hands and feet in the position of the normal infant. Again, as with the previous infant, the rapidity and degree of recovery of motor function was surprising. No determinations of the recovery of sensory function could be made. At the end of a week the animal walked and climbed. At the end of a month it moved accurately and rapidly, using both hands and both feet equally; the right hand and

foot, however, were somewhat less accurate than the left. Forced grasping disappeared at the same time on the two sides. Four months after operation only a slight exaggeration and awkwardness of the movements of the right side distinguished this animal from a normal infant.

2. *Bilateral motor and premotor.* The motor and premotor areas were later removed from the second hemisphere of the first-mentioned infant at the age of five months (first operation when 10 days old). The recovery of voluntary power was immediate. Within a few hours of operation the animal showed the postural characteristics of the bilateral motor-premotor animal of Bieber and Fulton (5). It lay on one side with undermost limbs extended and uppermost flexed, reflex grasping appeared strongly in all four extremities and was to change in intensity with change in position of the animal. From the first day it was able to right itself when lying on either side and also to reach for and grasp objects voluntarily with the right extremities. After several days it could perform voluntary movements on the left side also and recovery thereafter was extraordinarily rapid and complete.

At the end of the first week after operation it could walk and climb and feed itself by approximating its mouth to the food rather than by using its hands. It climbed rapidly and fairly accurately, sometimes slipping on flat surfaces, and always progressing on a broad base; there was hypermetria and movements were less well performed on the left. At the end of one month the difference between the two sides had about disappeared and there had been great improvement in the skill and accuracy of movements. The animal now fed itself with its hands, climbing continued to be executed better than walking, and while the intensity of forced grasping had diminished, the tendency to climb and cling persisted. It was sometimes unable to detach itself from the bars of the cage and would cling for hours if not removed.

During the next four months the animal had grown and developed in a healthy normal fashion. Its motor performance had become so adequate that it might easily have passed for normal. However, a definite motor deficit was still present, and movements were slower than normal; when placed in a cage with two slightly smaller animals it was unable to hold its own with the more agile cage-mates. The walking movements were still hypermetric and the animal developed a gallop, which was like the hopping of a rabbit. There was no evidence of forced grasping, except that the animal clung for hours to the breast of a larger monkey in the manner of the new-born infant. It exhibited the usual curiosity of the monkey, running and climbing everywhere when set free in a room. It climbed awkwardly and in jumping from chair to desk, a distance of perhaps 5 feet, it frequently missed its aim and fell to the floor. High stepping and hyperextension of the extremities were also present, although on passive manipu-

lation rigidity was not noticeable. The animal is still alive, 18 months after its last operation, and its neurological condition is unchanged (see fig. 3).

Discussion. That the recovery of motor function after extirpation of excitable motor areas in these monkeys is influenced by the remaining cortical areas is consistent with the observations of numerous earlier investigators who have affirmed the presence of an influence from various contralateral and ipsilateral cortical motor areas. Foerster (10) has shown in human beings that, after destruction of the precentral cortex, there is recovery of voluntary motor power which is integrated by extrapyramidal cortical areas of the same hemisphere. He also demonstrated the influence of the hemisphere ipsilateral to the paresis in a case in which a degree of voluntary movement was restored to the hemiplegic fingers, but occurred only with simultaneous movements of the normal hand. Gardiner (9) reports the case of a woman who, after the removal of an *entire hemisphere*, was able to move the contralateral lower extremity. Wertheimer and LePage (6) have shown in dogs, and Fulton (7) in monkeys and chimpanzees, that lesions of one hemisphere affect the ipsilateral as well as the contralateral extremities. These facts are consistent with the presence of ipsilateral fibers found in the pyramidal tracts. The fact that in older adult monkeys a greater permanent deficit is maintained than in younger individuals might also be expected since it is known clinically that younger people and especially children, recover more quickly than adults from hemiplegias.

The ability of one cortical area to assume the integration of *voluntary motor activity* to a greater or less degree after destruction of another motor area normally responsible for such integration seems, thus, well established. However, Jacobsen (11) working on psychological tests with these same animals, and with others from which only the frontal areas had been extirpated, has found that there are functions which, unlike voluntary purposeful movement, can be entirely and permanently abolished by extirpations limited to *all* the frontal association areas. The faculty of immediate recall, that is, the ability of an animal to make a correct choice after a given time interval which is normally present in the monkey, is, after extirpation of *all* of both frontal areas, entirely and permanently lost, *both in infants and in adult animals.*

The faculty of immediate recall can, then, be integrated only in one specific cortical area, but the function of voluntary purposeful movement, most perfectly integrated in area 4, can be imperfectly assumed by other cortical areas. In the older animals in which motor performance is normally integrated at cortical levels, interruption of all these pathways is followed by partial recovery of function which, although minimal, is present under certain conditions even after removal of all excitable cortex.

In the infants voluntary motor control after extirpation of all excitable tissue is entirely adequate to maintain the existence of the individual.

The question of which centers assume control of movement in these infants becomes of paramount interest. For various reasons it seems possible that these functions are assumed at subcortical levels. Clinical and experimental evidence indicates that subcortical centers,—the striate bodies, the cerebellum, influence motor performance. In the infant monkeys the pyramidal tracts are not yet developed and the relatively simple and uncoördinated movements of the animal at this age are, therefore, also integrated at a subcortical level. At such a time, then, unilateral and even bilateral removal of the excitable areas of the cortex can have little effect on motor performance, and the lower centers, functioning normally at this time, are able, as the animal matures, to integrate a remarkably accurate and well coördinated motor performance.

SUMMARY

Recovery of motor power in the extremities of monkeys after unilateral and bilateral ablations of the motor and premotor areas of the cortex, is influenced by a number of factors.

1. The age of the animals affects both rate of recovery and degree of motor deficit. Young and immature animals recover more quickly and extensively than adults.

2. The size of the lesion made in a single hemisphere also affects recovery, which is much more rapid after smaller lesions even though the smaller extirpations may include all excitable motor tissue for a given extremity.

3. The hemisphere ipsilateral to the extremities also influences recovery since extirpation from the motor areas of this side cause additional ipsilateral deficit.

4. In older animals when the interval between successive ablations of the motor-premotor areas of the two hemispheres exceeds four weeks there is some recovery. This slight return of voluntary movement occurs on the side that was first paralyzed.

5. In infant monkeys operated upon when forced grasping is normally present and when motor performance is still poorly coördinated, removal of the premotor and motor areas of one side has little effect on motor performance.

6. In such an infant extirpation of the motor areas of the second hemisphere after complete recovery from the first operation produces a permanent deficit, but voluntary movements are adequately performed, so that the animal is able to maintain itself, and to grow and develop normally.

7. The motor performance of such an animal, when well-grown (two

years of age) resembles that of the infant macaque. Forced grasping persists and movements are slow and incoördinated.

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DIET IN RELATION TO REPRODUCTION AND REARING OF YOUNG

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Guest, Nelson, Parks and Fulmer (1926) have demonstrated that rats grow at the normal rate with different single foods as the only source of vitamin B. Reproduction was approximately normal on these diets, but there was a high mortality of the young. Furthermore, a large number of females died during pregnancy or parturition on these diets, the mortality varying with the nature of the seed used as a source of vitamin B. The authors stated, "The abnormal results are not due to a deficiency of fat soluble vitamins but may be due to a deficiency of vitamin B. If such is the case, then it is very probable that the human being is close to the borderline as regards the needs for vitamin B in relation to lactation." At that time little was known of the complexity of the B factor. Wilkinson and Nelson (1931) subsequently showed that rats grow at a normal rate with 10 to 73.3 per cent of soy bean as the only source of vitamins B and G. However, satisfactory lactation was not obtained on any level of soy bean investigated. Different animal organs were then studied from the standpoint of lactation potency; and it was found that liver from hogs and cattle and hog kidney markedly improved lactation, when added to the different diets. The lactating factor was destroyed in an electric oven at 120°C. Due to the fact that liver is a rich source of vitamin G, it seemed worth while to ascertain whether the above products failed in lactation because of deficiency of this factor or whether a specific substance is required for lactation.

EXPERIMENTAL. All of the experiments were performed on rats. The rations employed in all of the lactation studies recorded in the first four tables consisted of casein 18 per cent, salt mixture 3.7 per cent, butterfat 4.0 per cent, cod liver oil 1.0 per cent, varying amounts of food materials or vitamin preparations furnishing vitamins B and G, and dextrin to one hundred per cent. Female rats on these experiments were transferred from the stock ration to the experimental rations at the time of parturition. The data in table 5 were obtained from animals receiving the basal food mixture composed of casein, salts, cod liver oil and dextrin plus wheat germ oil; and they were reared on this ration.

The animals were placed on shavings for seven days following parturition and then on two mesh per inch screens until weaned. Females were allowed uniform litters of six young each; the young were weaned at 28 days of age. The mode of preparation of the various components of the basal ration is given in a previous paper (1926).

The data in table 1 show the results obtained on various food materials as the sole source of vitamins B and G. The per cent mortality of the young was in practically all cases very high. Only in a few instances was the weaning weight of the young normal; and it is to be observed that a high weaning weight is not necessarily accompanied by a low mortality of the young. Table 2 shows the effect of addition of autoclaved yeast to the products given in table 1. The autoclaved yeast was prepared by moistening Fleischmann's dried yeast with distilled water to make a thick paste, autoclaving for five hours at 15 pounds pressure and

TABLE 1

Lactation on diets containing various seeds and products from seeds

SOURCE OF VITAMINS B AND G		NUMBER OF LITTERS	NUMBER OF YOUNG	NUMBER OF YOUNG WEANED	AVERAGE WEANING WEIGHT	PER CENT MORTALITY
Oatmeal	60	8	48	40	45.2	16.6
Yellow corn	60	12	72	20	36.0	72.2
White corn	60	12	72	27	41.1	62.5
Barley	60	12	72	47	38.1	34.7
Wheat	30	6	36	16	34.2	55.5
Rice polishings	10	12	72	50	39.5	30.5
Wheat germ	10	12	72	27	47.4	62.5
Rice bran	10	12	72	40	46.7	44.4
Corn germ	10	12	72	37	22.5	48.6

drying at 80°C. The results in table 2 are self-explanatory. They emphasize the point that autoclaved yeast markedly reduces mortality of the young and enhances the weaning weight of the young from the mothers on the various seeds, and products from seeds, that were studied. An exception is to be observed in the experiment on corn germ, in which case the addition of 10 per cent of autoclaved yeast reduced the mortality only moderately and had no effect on the weaning weight of the young. The autoclaved yeast replaced an equivalent amount of dextrin in the ration. Since autoclaved yeast contains no vitamin B, it is evident that the factor, or factors, responsible for this favorable effect reside in the G fraction of the yeast.

Following this work preparations of vitamins B and G were made and the effect of these preparations on lactation studied. The results are recorded in table 3. Fraction 8B is an activated fuller's earth prepara-

tion of vitamin B, which was shown by assay not to contain vitamin G. This preparation was made from rice polishings by the following method:

TABLE 2

Effect of autoclaved yeast when added to basal ration containing seeds or their products as sources of vitamins B and G

SOURCE OF VITAMINS B AND G	NUMBER OF LITTERS	NUMBER OF YOUNG	NUMBER OF YOUNG WEANED	AVERAGE WEANING WEIGHT	PER CENT MORTALITY
Oatmeal 60 Yeast auto 5	6	36	30	49.0	16.6
Oatmeal 60 Yeast auto 10	8	48	48	51.3	0.0
Yellow corn 60 Yeast auto 5	12	72	65	48.4	9.7
Yellow corn 60 Yeast auto 10	12	72	65	54.1	9.7
White corn 60 Yeast auto 5	12	72	66	55.2	8.3
White corn 60 Yeast auto 10	12	72	65	57.1	9.7
Barley 60 Yeast auto 5	12	72	64	44.7	11.1
Barley 60 Yeast auto 10	12	72	66	54.8	8.3
Wheat 30 Yeast auto 10	6	36	30	50.0	16.6
Rice polishings 10 Yeast auto 10	12	72	70	56.3	2.7
Wheat germ 10 Yeast auto 10	12	72	66	56.8	8.3
Rice bran 10 Yeast auto 10	12	72	72	54.1	0.0
Corn germ 10 Yeast auto 10	12	72	44	27.2	38.8

4 kilograms of rice polishings were stirred intermittently through the day in a battery jar with 95 per cent ethyl alcohol, containing 30 ml. glacial

acetic acid to 10 liters of alcohol. After 24 hours the supernatant liquid was siphoned into another battery jar containing four kilos of rice polishings. A battery of four jars was used and a new jar of rice polishings added each day; and the extract from the new jar of the day before was removed and concentrated in vacuo, so that the temperature remained below 40°C. Fat was removed from the concentrate in a separatory

TABLE 3

Effect of preparations of vitamins B and G on lactation

LOT NUMBER	SOURCES OF VITAMINS B AND G	NUMBER OF LITTERS	NUMBER OF YOUNG	NUMBER OF YOUNG WEANED	AVERAGE WEIGHT AT WEANING	PER CENT MORTALITY
189	A.F.E. 8B 0.053 g.	4	24	0	0	100
274	A.F.E. 8B 0.318 g.	12	72	50	20.5	30.5
294	A.F.E. 8B 0.318 g.	12	72	44	19.3	38.8
296	H.L.P. 11F$_1$ 1.66 g.	12	72	35	18.6	51.3
193	D.H.L. 11A 15%	12	72	17	26.8	76.4
203	D.H.L. 11A 3.3%	6	36	1	18.0	97.2
202	Yeast auto 10%	6	36	12	17.5	66.6
192	A.F.E. 8B 0.053 g. D.H.L. 11A 0.20 g.	6	36	1	28.0	97.2
244	A.F.E. 8B 0.106 g. D.H.L. 11A 0.500 g.	10	60	34	30.7	43.3
245	A.F.E. 8B 0.212 g. D.H.L. 11A 0.500 g.	10	60	33	28.9	44.9
246	A.F.E. 8B 0.106 g. D.H.L. 11A 1.0 g.	10	60	4	29.0	93.3
269	A.F.E. 8B 0.212 g. D.H.L. 11A 1.0 g.	12	72	40	31.9	44.4
270	A.F.E. 8B 0.318 g. D.H.L. 11A 1.0 g.	12	72	58	45.2	19.4
272	A.F.E. 8B 0.318 g. Yeast auto 10%	12	72	60	35.2	16.6
273	A.F.E. 8B 0.318 g. H.L.P. 11F 1.33 g.	12	72	67	31.2	6.9
297	A.F.E. 8B 0.318 g. H.L.P. 11F$_1$ 1.66 g.	12	72	66	39.3	8.3

funnel, the concentrate transferred to a flask, and neutral 25 per cent lead acetate added to give the maximum precipitate. The lead was removed from the filtrate by means of 20 per cent H_2SO_4. The $PbSO_4$ was filtered, and the acidity of the lead free filtrate was adjusted to pH 4.0 to 4.5; 75 grams of fuller's earth for each 4 kilos of rice polishings were added, the acidity readjusted to pH 4.0 to 4.5, and the mixture stirred for 3 hours. The filtrate was again adjusted to a pH of between 4.0 and 4.5 and 20

grams of fuller's earth added for each 4 kilos of rice polishings; following which the pH was again restored to between 4.0 and 4.5 and the mixture stirred for 3 hours. The combined preparations were first air dried and then dried further in a vacuum desiccator over $CaCl_2$; and they were finally dried in a vacuum oven at 40°C. for 12 hours. This preparation is known as the activated fuller's earth preparation, and in the table is designated as A.F.E. 8B.

H.L.P. in table 3 refers to hog liver preparation. This preparation was made as follows: fresh hog liver was minced in a food chopper and then stirred into two liters of boiling water per kilo of liver; this mixture was boiled for 3 minutes and filtered through a Buchner funnel; and the insoluble material was washed with 1 liter of hot water per kilo of liver. The extracts and washings were concentrated under reduced pressure (temp. 50°C.) to a volume of 150 ml., cooled, and 850 ml. absolute alcohol added; the precipitate which formed was dissolved in water and dried on dextrin. One gram of preparation 11F corresponds to 3 grams of hog liver, and 1 gram of $11F_1$ corresponds to 6 grams of hog liver. D.H.L. refers to dried hog liver prepared by drying minced hog liver in an oven at 80°C. Autoclaved yeast was prepared as for experiments in table 1.

The data shown in table 3 were obtained from pregnant females transferred from the growing ration at the time of parturition. The results show that a supply of either vitamins B or G alone failed to support lactation, indicating that neither one of these vitamins is stored in sufficient amounts by the females, while on the growing ration, to promote successful rearing of young, when the mothers are transferred to the experimental diet. The data, furthermore, reveal that dried hog liver, although rich in G, did not give good results for lactation on the levels used (3.3 and 15 per cent). The best results on mortality were obtained by combining the activated fuller's earth and hog liver preparations (lots 273 and 297). The best weaning weight is shown in lot 270, where the animals received activated fuller's earth preparation plus dried hog liver. The young, on autoclaved yeast, dried hog liver 11A, and hog liver preparation $11F_1$, when examined between the second and third week of life revealed empty stomachs—indicating failure of milk secretion. The animals at this time had convulsions, were very excited, screamed and jumped about the cages in a queer manner. They then died. The animals receiving the A.F.E. 8B preparation when autopsied showed milk and curd in the stomach. They did not show the characteristic spasms and excitement of the above group. They ceased to grow and died.

Assay of preparations 11F and $11F_1$ showed that these materials contained no vitamin B. Cane sugar was used in the diets as the sole source of carbohydrates in the assays for B and G, since it was found that some starches and dextrins contain vitamin G.

The next step was to ascertain the effect of fractions 8B and 11F on lactation when added to 60 per cent yellow corn and 60 per cent wheat as the only sources of B and G. The results are given in table 4. The mortality of the young was not reduced by the addition of 0.318 gram of fraction 8B daily to the yellow corn ration; but it was reduced markedly by the addition of 1.66 grams of fraction 11F daily to the above ration; and, furthermore, the weight of the young at weaning was markedly increased. This shows that 60 per cent of yellow corn as the sole source of vitamins B and G contains enough B for lactation but does not contain sufficient vitamin G. The addition of 0.318 gram of fraction 8B to 60 per cent of wheat as the sole source of vitamins B and G had only a slight effect on the mortality and no effect on the weight of the young when

TABLE 4

Effect of addition of preparations of vitamins B and G to basal food mixture containing yellow corn or wheat as the sole sources of B and G

SOURCES OF VITAMINS B AND G	NUMBER OF LITTERS	NUMBER OF YOUNG	NUMBER OF YOUNG WEANED	AVERAGE WEANING WEIGHT	PER CENT MORTALITY
Yellow corn 60	5	30	10	27.5	66.6
Yellow corn 60 0.318 g. fraction 8B	6	36	4	30.2	88.8
Yellow corn 60 1.66 g. fraction 11F	6	36	32	54.5	11.1
Wheat 60	6	36	16	27.5	55.5
Wheat 60 0.318 g. fraction 8B	6	36	22	29.8	38.8
Wheat 60 1.66 g. fraction 11F	6	36	36	53.8	0.0

weaned. However, the addition of 1.66 grams of fraction 11F to 60 per cent of wheat as the only source of vitamins B and G reduced the mortality of the young to 0 and increased the weaning weight by more than 90 per cent.

Having shown that vitamin G markedly supplements corn and wheat for lactation, whereas vitamin B does not, we next studied the effect of B and G preparations as the only source of vitamins B and G on growth, reproduction, and lactation. The results are given in table 5. The synthetic basal diet of these animals was the same as the first group of animals received, except that 1 per cent of wheat germ oil replaced an equivalent amount of dextrin, in order to supply the reproductive factor vitamin E. The animals recorded in table 5 received their experimental rations at weaning and they were carried through successive generations. The mortality on most of these diets was very high. On the lower levels of

the B and G preparations the weaning weights were low; whereas, on the higher levels of these preparations, the weights of the young at weaning were much greater. However, the weights of the young were less and mortality greater than that obtained by supplementing natural foods (corn or wheat) with vitamin G preparation 11F. This would seem to

TABLE 5

Effect of preparations of vitamins B and G on reproduction and lactation

LOT NUMBER	SOURCES OF VITAMINS B AND G DURING LACTATION*	NUMBER OF LITTERS	NUMBER OF YOUNG WITH MOTHERS	NUMBER OF YOUNG WEANED	AVERAGE WEIGHT AT WEANING	PER CENT MORTALITY	NUMBER OF YOUNG BORN DEAD	NUMBER OF FEMALES ON EXPERIMENT
307	A.F.E. 8B 0.15 g. H.L.P. 11F$_1$ 1.0 g.	16	85	14	30.6	83.5	0	4
307 2nd gen.	A.F.E. 8B 0.15 g. H.L.P. 11F$_1$ 1.0 g.	7	38	24	32.5	37.1	4	3
307 3rd gen.	A.F.E. 8B 0.15 g. H.L.P. 11F$_1$ 1.0 g.	2	4	0		100		7
308	A.F.E. 8B 0.30 g. H.L.P. 11F$_1$ 2.0 g.	12	56	19	44.0	66.0	0	4
308 2nd gen.	A.F.E. 8B 0.30 g. H.L.P. 11F$_1$ 2.0 g.	18	87	19	43.7	78.1	7	7
308 3rd gen.	A.F.E. 8B 0.30 g. H.L.P. 11F$_1$ 2.0 g.	3	12	0		100		6
321	A.F.E. 8B 0.3 g. H.L.P. 11F$_1$ 2.0 g.	5	19	4	45.2	78.9	11	4
321$_a$	A.F.E. 8B 0.6 g. H.L.P. 11F$_1$ 4.0 g.	6	32	15	42.4	53.1	3	4

* Females in lot 307 received A.F.E. 8B 0.025 gram, H.L.P. 0.166 gram and those in lots 308, 321 and 321a received A.F.E. 8B 0.05 gram, H.L.P. 0.33 gram between lactation periods.

indicate that another factor aside from vitamins B and G is associated with milk secretion and rearing of young. Fraction A.F.E. 8B, at a level of 0.025 gram plus 0.166 gram of H.L.P. 11F$_1$ gave normal growth; so that the lowest levels of these preparations recorded in table 5 are 6 times the amount for normal growth. The highest levels of preparations B and G recorded in table 4 are 24 times the amount required for normal growth;

and still lot 321a showed a mortality of 53.1 per cent. Although not indicated in table 5 the females reproduced normally.

SUMMARY

1. The mortality of young rats is high on rations containing oatmeal, yellow corn, white corn, barley, wheat, rice polishings, wheat germ, rice bran or corn germ as the sole source of vitamins B and G.

2. Autoclaved yeast markedly reduces the mortality on these diets and increases the weaning weight of the young.

3. Preparations of vitamins B and G have been administered to lactating animals, and it has been observed that lactation was unsuccessful when a preparation of B or G was fed alone.

4. Combinations of vitamins B and G preparations gave better results than either one alone; and of the preparations studied, the lowest mortality of the young was obtained with a combination of activated fuller's earth preparation of vitamin B plus hog liver preparation.

5. Vitamin G preparation markedly reduces mortality of young rats, and increases the weaning weight when administered to females receiving 60 per cent of wheat or 60 per cent of yellow corn as the sole source of vitamins B and G. Vitamin B preparation did not have this effect.

6. Data are presented on the effect of purified B and G preparations on growth, reproduction, and lactation; and from these studies it appears probable that a new factor may be necessary for lactation.

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INCREASED SALT APPETITE IN ADRENALECTOMIZED RATS

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Adrenalectomized rats are known to have a definitely increased salt need, inasmuch as the administration of large quantities of salt either greatly reduces or eliminates the symptoms of insufficiency, and actually increases the survival incidence (Rubin and Krick, 1933; Gaunt, Tobin and Gaunt, 1935; Kutz, McKeown and Selye, 1934). But is this need accompanied by an increased salt appetite, and will the adrenalectomized rat, if given free access to salt take sufficient quantity to keep itself alive and free from symptoms of insufficiency? In an effort to answer these questions the following experiments were undertaken.

METHODS. It was necessary for this purpose to devise a method by which salt could be given freely, under circumstances which made possible measurement of the actual amount ingested. It would have been simplest, of course, to present granular salt in a separate container. However, the small amount ingested daily and the comparatively large amount spilled or carried away on the paws definitely ruled out a quantitative measure by this method. For this reason salt was offered in solution in the drinking water. At the outset a 1 per cent salt solution was used because Rubin and Krick obtained their successful results from a solution of approximately this strength; but since, with only the salt solution available, the rat's intake would depend partly on the salt need and partly on the thirst for water, access was given at the same time to tap water presented in a second container. In this way the rat could satisfy its thirst and its salt appetite independently.

In the first series of experiments with the 1 per cent salt solution and water, the animals also received the usual amount of salt in the McCallum diet (approximately 0.145 gm. per day). Inasmuch as the normal rats did not distinguish between the salt solution and the water, a second series of experiments was started with the strength of the salt solution increased to 3 per cent and no salt given in the food. It was found then that the normal animals did differentiate very definitely between the 3 per cent salt solution and the water.

The rats were kept in individual cages containing a food cup, and two inverted graduated bottles, one with salt solution, the other with tap

water. The water and salt solutions were changed at the same time to avoid any difference in the freshness of the two liquids. The fluid intake was recorded daily and the body weight was recorded weekly.

To establish the effects produced by the salt solution two control groups of animals were adrenalectomized and kept under exactly the same conditions, except that one received only the salt contained in the food, while the other received no salt, either in the drinking water or in the food.

The adrenalectomy was done by the technique in which the surrounding fat, connective tissue and also about one-quarter inch of the pedicle are removed with the gland (Pencharz, Olmsted and Giragossintz, 1931; Firor and Grollman, 1933). In order to make certain that the effects produced depended specifically on adrenalectomy, a third group of control experiments was performed to determine the relation of gonadectomy and hypophysectomy to salt appetite.

RESULTS. *Survival rate of adrenalectomized rats on a saltless diet and on a standard McCollum diet.* Fifteen animals that had previously been on the standard McCollum diet were adrenalectomized and placed on a saltless diet. All of these animals immediately developed symptoms of insufficiency with a loss of weight, appetite, and death after an average of 11 days. None of the animals survived.

Twenty-six animals raised on the McCollum mixture were adrenalectomized and continued on this standard diet from which they received approximately 0.145 gram of salt per day. Sixteen of these animals died at an average interval of 17 days, while ten animals, or 39 per cent, gave signs of living their normal span of life, and were killed about forty-five days after adrenalectomy.

Increased survival rate of adrenalectomized rats with access to 1 per cent or 3 per cent salt solution and tap water. Thirteen animals on the regular McCollum diet given the choice of drinking 1 per cent salt solution or tap water, drank enough of the former to increase very markedly their chances of survival. Nine, or 69 per cent, showed all signs of living indefinitely and only four, or 31 per cent, died. This is a marked increase in the survival rate over that of the animals which received only the salt contained in the McCollum diet. Moreover the average duration of life of the animals that died was 19 days, which is greater than that of animals on either saltless diet or on only the McCollum mixture.

Five animals given the choice of 3 per cent salt solution or tap water showed a survival rate of 80 per cent. One animal lived 38 days, the others showed a normal gain in body weight and no signs of insufficiency except a slightly decreased appetite.

Further proof that the animals were actually kept alive by the salt which they ingested voluntarily, is shown by the effects produced on survival when the 1 per cent salt solution was removed leaving only tap

water available. The nine animals which gave every indication of living indefinitely on the 1 per cent salt solution were deprived of the salt solution at intervals ranging from 33 to 77 days after adrenalectomy but were still kept on the McCollum diet. (See the last two columns in table 1.) Eight out of the nine animals died after an average of 5.1 days with a range of variation of 4 to 7 days, while the ninth animal was still alive on the 36th day.

Amount of increase of salt intake in adrenalectomized rats. A marked effect produced by adrenalectomy on the salt appetite is shown very clearly in figure 1. It will be seen that before adrenalectomy the tap water averaged 20 cc. per day, the intake of 1 per cent salt solution only 10 cc. per day; and that almost immediately after adrenalectomy the intake of tap water began to decrease, while the intake of salt solution showed a

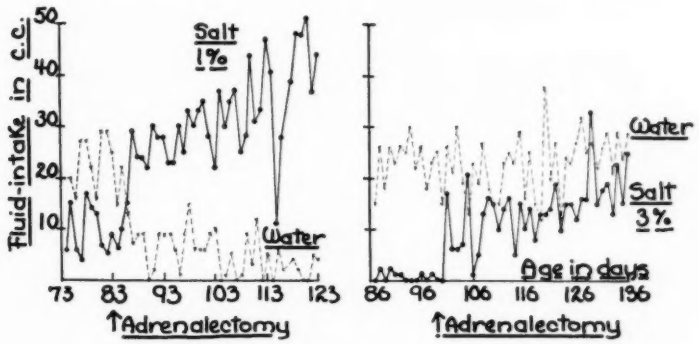

Fig. 1

sharp increase. Forty days after adrenalectomy, when the animal was killed, the salt solution intake had reached a level of 45 cc. per day and was still increasing while the water intake had decreased to less than 4 cc. per day.

The results of these experiments are summarized in table 1. It will be seen that the average daily intake of tap water decreased from 16.5 cc. for the 10 days before adrenalectomy to 3.4 cc. for the 20 to 30 day period after adrenalectomy, while the intake of the 1 per cent salt solution increased from 16.9 cc. for the 10 day pre-operative period to 27.6 cc. for the 20 to 30 day post-operative period. These daily averages of 27.6 cc. of salt solution and 3.4 cc. of tap water indicate definitely that the animals differentiated between these two fluids. However, before adrenalectomy the average daily intake was practically identical for tap water and for the 1 per cent salt solution (16.5 cc. as compared to 16.9 cc., respectively). This must mean that before operation the animals did not

differentiate between the two fluids. In keeping with this view is the fact that some of the animals drank more water while others took more salt solution, a fact which suggests that it was largely a matter of chance which bottle was selected. It seems probable that had a definite distinction been made between these two fluids, the salt solution intake before adrenalectomy would have been much lower, consequently the percentage increase in the salt need after adrenalectomy would have been much greater than was found in these experiments.

TABLE 1

Choice of 1 per cent salt solution or water (dry diet containing 1 per cent salt)

RAT NO.	AGE	AVERAGE DAILY TAP WATER INTAKE		AVERAGE DAILY INTAKE OF 1% SALT SOLUTION		AVERAGE DAILY SALT INTAKE FROM 1% SALT SOLUTION		AVERAGE DAILY SALT INTAKE IN FOOD		TOTAL AVERAGE DAILY SALT INTAKE		BODY WEIGHT		AVERAGE DAILY SALT INTAKE PER KILOGRAM BODY WEIGHT		SURVIVAL	
		10 days before adrenalectomy	20-30 days after adrenalectomy	10 days before adrenalectomy	20-30 days after adrenalectomy	10 days before adrenalectomy	20-30 days after adrenalectomy	10 days before adrenalectomy	20-30 days after adrenalectomy	10 days before adrenalectomy	20-30 days after adrenalectomy	Day of adrenalectomy	30th day after adrenalectomy	10 days before adrenalectomy	20-30 days after adrenalectomy	After adrenalectomy	After salt removal
	days	*cc.*	*cc.*	*cc.*	*cc.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*days*	*days*
1	78	3.0		7.9		0.079		0.120		0.199		145	140	1.45		15 D†	
2	78	15.1		19.9		0.199		0.120		0.319		150	141	2.13		16 D	
3	78	14.9		11.9		0.119		0.120		0.239		145	125	1.65		14 D	
4	83	16.4	4.7	10.8	33.2	0.108	0.332	0.120	0.110	0.228	0.442	165	173	1.38	2.55	33 +	4 D
5	98	26.3	4.8	35.1	44.6	0.351	0.446	0.121	0.096	0.470	0.552	214	230	1.67	2.40	34 +	5 D
6	67	15.6	1.6	10.0	21.4	0.100	0.214	0.100	0.153	0.320	0.367	188	207	1.70	1.77	77 +	36 K*
7	80	19.4	1.4	6.7	19.3	0.067	0.193	0.143	0.110	0.210	0.303	220	223	0.95	1.36	40 +	4 D
8	83	12.2	1.9	37.7	26.7	0.377	0.267	0.120	0.110	0.497	0.377	170	181	2.92	2.08	49 +	7 D
9	83	22.6	3.6	8.9	35.0	0.089	0.350	0.120	0.110	0.209	0.460	170	180	1.23	2.55	47 +	6 D
10	89	23.0	4.5	9.1	27.0	0.091	0.270	0.120	0.110	0.211	0.380	182	195	1.16	1.95	48 +	6 D
11	82	9.2	3.1	19.3	19.6	0.193	0.196	0.107	0.093	0.293	0.295	151	168	1.94	1.75	42 +	5 D
12	73	30.6	6.9	5.7	25.5	0.057	0.255	0.163	0.099	0.220	0.354	234	215	0.94	1.64	31 D	
13	64	6.6	1.2	37.4	24.1	0.374	0.241	0.120	0.110	0.494	0.351	217	205	2.28	1.71	46 +	4 D
Av.	80	16.5	3.4	16.9	27.6	0.169	0.276	0.123	0.110	0.301	0.388	181	183	1.65	1.98		

* Killed.
† Died.

The failure of the rats before operation to distinguish between the tap water and the 1 per cent salt solution may have been due either to the fact that the salt solution was too weak or to the fact that the salt which they received in the diet was sufficient to satisfy the salt need as well as to dull the sensitivity of the salt appetite.

Because of this failure of the animals to differentiate definitely between the 1 per cent salt solution and water before adrenalectomy, a 3 per cent salt solution was substituted. This was not too concentrated to be measured accurately in the water bottles used in these experiments, and it

was of sufficient strength that the five animals which had the choice of drinking it or tap water differentiated between the two before adrenalectomy as well as afterwards. A record from one of these animals is presented in figure 1. It will be seen that the intake of the 3 per cent salt solution increased from a level of less than 2 cc. per day before adrenalectomy to 20 cc. afterwards, while the water intake remained practically the same.

The results as summarized in table 2 show that before adrenalectomy the average daily water-intake was 23.0 cc. while the intake of 3 per cent salt solution was 2.2 cc. It will be noted that this marked difference in the consumption of salt solution and water was present in all five animals. The intake of the 3 per cent salt solution for the 20 to 30 day period after

TABLE 2

Choice of 3 per cent salt solution or water (dry diet without salt)

RAT NO.	AGE	AVERAGE DAILY TAP WATER INTAKE		AVERAGE DAILY INTAKE OF 3% SALT SOLUTION		AVERAGE DAILY SALT INTAKE FROM 3% SALT SOLUTION		BODY WEIGHT		AVERAGE DAILY SALT INTAKE PER KILOGRAM BODY WEIGHT		SURVIVAL
		10 days before adrenalectomy	20–30 days after adrenalectomy	10 days before adrenalectomy	20–30 days after adrenalectomy	10 days before adrenalectomy	20–30 days after adrenalectomy	Day of adrenalectomy	30th day after adrenalectomy	10 days before adrenalectomy	20–30 days after adrenalectomy	
	days	*cc.*	*cc.*	*cc.*	*cc*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*days*
14	99	24.4	23.9	0.6	16.5	0.018	0.495	383	370	0.047	1.33	65 K
15	93	10.0	14.1	1.8	4.1	0.054	0.123	162	174	0.341	0.71	67 K
16	93	17.0	16.7	2.7	11.4	0.081	0.342	170	175	0.476	1.98	38 D
17	91	24.6	20.3	3.0	18.1	0.090	0.543	265	268	0.353	2.04	65 K
18	91	39.0	27.7	2.7	12.5	0.081	0.375	292	318	0.284	1.19	65 K
Av.	93	23.0	20.5	2.2	12.5	0.065	0.376	254	261	0.300	1.45	

adrenalectomy was 12.5 cc., almost six times as high as the intake before. This, undoubtedly, is a much more correct estimate of the salt needs of the adrenalectomized animals than was obtained in the experiments with the 1 per cent salt solution.

Effect of gonadectomy and hypophysectomy on salt appetite. It was found that gonadectomy and hypophysectomy have no effect on salt appetite. The average daily intake of 3 per cent salt solution of four gonadectomized rats was 3.0 cc. for the 10 days before adrenalectomy and 3.9 cc. for the 20 to 30 days after; and for the six hypophysectomized rats it was 3.6 cc. before and 1.5 cc. afterwards, a decrease proportional to the general decrease in metabolism.

It may be assumed, then, that the increased salt appetite which follows adrenalectomy is specific for the deficiency created by the loss of the

secretions of the adrenal gland. It is of interest that the atrophy of the adrenals found in the hypophysectomized rats was not associated with an increase in salt appetite.

Salt appetite of normal rats. In the above experiments it was shown that the amount of salt taken voluntarily by adrenalectomized rats gave an indication of their salt need. It seemed likely that the voluntary salt intake could also be a measure of the salt need of normal animals. It was of interest to know, then, how the salt need determined in this way compares with the salt that the animals receive in the standard McCollum diet.

This diet contains 1 per cent salt which according to the calculations of Wang (1925), of an average daily food intake of 14.5 gram in adult rats would mean an average daily salt intake of 0.145 gram or 0.659 gram per kilogram body weight.

Records taken on a group of nineteen normal animals on a saltless diet with a choice of either 3 per cent salt solution or tap water gave a daily voluntary salt intake of 0.123 gram or 0.577 gram per kilogram body weight, which is very nearly the same as the amount received in the McCollum diet. It was thus determined by a very different method that the salt present in the McCollum diet is an adequate amount for normal animals.

DISCUSSION. The fact that the salt appetite of adrenalectomized rats has such a close relationship to the salt deficiency indicates that appetite may be used as a measure of the deficiencies produced by endocrine disturbances, or by pathological changes in other parts of the body.

It has been observed on the medical wards of the Johns Hopkins Hospital that patients with Addison's disease have spontaneously expressed a great appetite for foods rich in salt, particularly ham and herring. It is also known that during pregnancy, when the greatest changes take place in the entire endocrine system, appetites may also change considerably. Thus it may be that even in man a closer study of the appetite might throw more light on the actual needs and deficiencies present in such conditions as pregnancy or in acute or chronic disturbances of the endocrine system.

SUMMARY

1. The survival rate of thirteen animals adrenalectomized and put on a saltless diet was zero per cent and the average length of life after adrenalectomy was 11 days.

2. The survival rate of twenty-six animals adrenalectomized and continued on the standard McCollum diet (approximately 0.145 gram of salt per day) was 39 per cent and the average duration of life of the animals that died was 17 days.

3. Thirteen rats kept on the standard diet and given the choice of tap

water or 1 per cent salt solution ingested a larger quantity of salt solution after adrenalectomy and their survival rate was increased to 69 per cent.

4. Five rats kept on a saltless diet but given the choice of a 3 per cent salt solution or tap water ingested six times as much salt solution after adrenalectomy and showed a survival rate of 80 per cent.

6. It was thus determined that the salt appetite is greatly increased by adrenalectomy and by virtue of this appetite the survival rate is also greatly increased.

7. The salt needs of the nineteen normal rats determined by the choice method on the 3 per cent salt solution was shown to be approximately the same as the amount calculated empirically by McCollum; that is, 0.577 gram per kilogram body weight per day as compared to 0.659 gram for the McCollum diet.

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THE EFFECT OF THYROID ADMINISTRATION UPON THE DIFFERENTIATING ABILITY OF DOGS

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The effects of oral administration of desiccated thyroid upon salivary conditioned reflexes in dogs were studied by Zawadowsky and his co-workers (1928–29) who reported an initial depression and a subsequent stimulation, and by Crisler and his co-workers (1933) who observed a stimulation followed by depression.

The present study was begun as part of an investigation into the physiology of sleep. In the attempt to determine the rôle of conditioned reflexes and of differentiation in the production of "internal inhibition" and sleep, it was thought desirable to have some agent which could be used to increase or decrease the differentiating ability at will. The reports of the workers cited above suggested that thyroid would be such an agent.

Because the conclusions arrived at by the several observers were to some extent at variance with each other, we found it necessary to reinvestigate the problem. Furthermore, since our interest was centered on the effects of thyroid upon central nervous system mechanisms, we decided to work with a motor rather than a secretory reflex so as to eliminate the possibility of direct action of the desiccated thyroid upon the responding organ.

METHODS. Each of five observers worked independently with one dog. The dogs were young, male mongrels, 8 to 12 kgm. in weight. Each dog was conditioned, by the usual method, to raise his left hind paw in response to the hitherto inadequate stimulus of a metronome sounded at a frequency of 100 beats per minute. The unconditioned stimulus was a tetanizing current passed through the left hind paw for about 0.02 minute. The current was of a strength that insured a flexion response of the corresponding leg in the particular dog. The dog was harnessed in a wooden frame. From an adjacent room the experimenter observed the reactions of the dog through a small glass window. A ruled board fastened to the wooden frame in back of the dog enabled the observer to determine by direct observation the height to which the dog lifted his paw.

When the dog showed perfect conditioning for three successive days (about 15 trials daily), the process of differentiation was begun. Thus, in our experiments, a metronome sounded at 100 beats per minute was the

positive conditioned stimulus. By not reinforcing a frequency of 200 metronome beats, this frequency was established as a stimulus to be differentiated from the 100 beats and not responded to (negative conditioned stimulus); this was alternated with the positive conditioned stimulus in irregular order. As the dog learned to differentiate perfectly between the positive and negative conditioned stimuli, the frequency of the latter was decreased so as to approach that of the positive stimulus. This procedure was repeated until for each dog the differentiation range was too small to permit of 100 per cent correct discrimination; *i.e.*, in a small percentage of the cases the dog would fail to respond to the positive metronome frequency and in some cases would respond to the negative frequency. Such a level of performance permits the detection of both improvement and deterioration under the influence of changed conditions.

At this point two-week periods of thyroid feeding were alternated with control periods of equal length. Two grams of thyroid in capsules were given orally each day during thyroid periods, but the dogs were tested on only 5 or 6 days each week. The percentages of correct responses to the positive conditioned and negative conditioned stimuli, as well as the average heights of the responses to the unconditioned, positive conditioned, and negative conditioned stimuli were computed.

RESULTS. Two dogs went through three control periods interspersed with two thyroid periods. One other dog went through two periods of each type. The results obtained on each of these three dogs are presented in graphic form in figures 1 to 4. The other two dogs were used for another investigation, but, when they reached the level of imperfect differentiation, were put through periods of thyroid feeding, and the results in each case were the same as in the first three dogs.

As would be expected, the breaking point at which differentiation was no longer perfect was not the same in different animals. In dog II it was a metronome rate of 112 beats per minute; in dog III, 118; in dog I, 130. This was reflected in the average magnitude of the conditioned response as compared to the unconditioned. As a rule, the conditioned response is weaker than the unconditioned (Hull, 1934), but we observed in addition that the better the differentiating ability of the dog the nearer did the average magnitude of the conditioned response approach that of the unconditioned. Thus in dog II the ratio was 0.92, in dog III, 0.79, and in dog I, only 0.39.

The effect of feeding two grams of thyroid daily was a definite improvement in the differentiating ability of the dogs. As can be seen from figures 1 and 2, not only was there an average higher percentage of correct responses during thyroid administration, but the percentage of correct responses to the negative conditioned stimulus was higher during the second half compared to the first half of thyroid feeding periods, while the

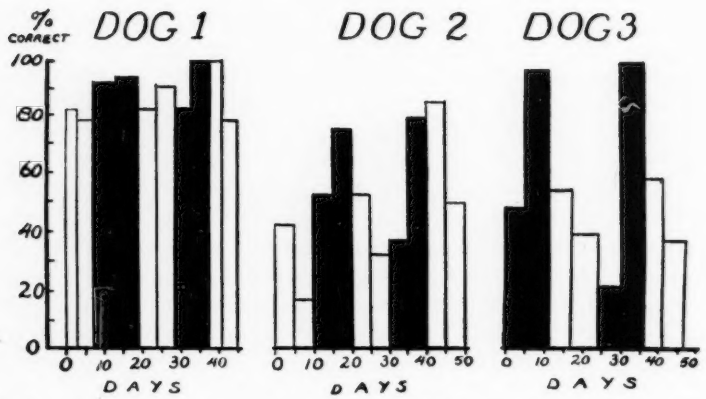

Fig. 1. Percentage of correct responses to inhibitory conditioned stimulus, a metronome rate of 130 per minute for dog I, 112 for dog II, and 118 for dog III. The correct response consisted of a *failure* to lift the left hind paw when the particular inhibitory metronome frequency was sounded. Black areas represent periods of thyroid feeding, white—interspersed control periods when no thyroid was given. The number of days indicated refer to days of testing, there being 5 or 6 such days per week.

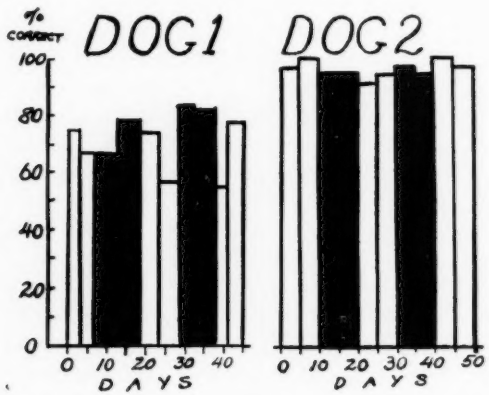

Fig. 2. Percentage of correct responses to positive conditioned stimulus, a metronome rate of 100 per minute in all cases. The correct response consisted of a lifting of the left hind paw, when the above metronome frequency was sounded. Black and white areas as in figure 1. Data for dog III were omitted because the percentages in all periods were close to 100.

opposite was true for the control periods. However, there was no change in the percentage of correct responses to the positive conditioned stimulus, under the influence of thyroid, in dog II, where these percentages were

all between 90 and 100, nor in dog III, where the percentages were close to 100.

The average magnitudes of the responses were also increased as a result of thyroid feeding, but this was true of the unconditioned as well as the conditioned responses (figs. 3 and 4).

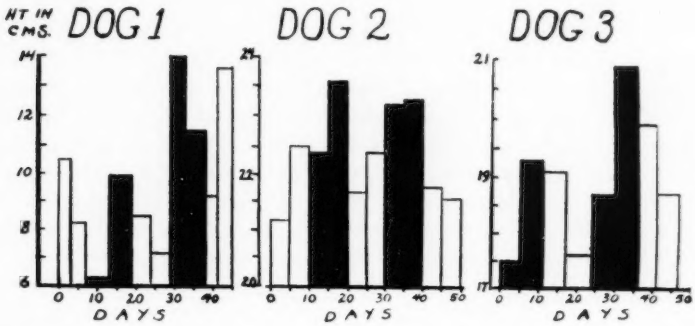

Fig. 3. The average height (in centimeters) to which the left hind paw was lifted in response to the positive conditioned stimulus, a metronome rate of 100 per minute. Black and white areas as in figure 1.

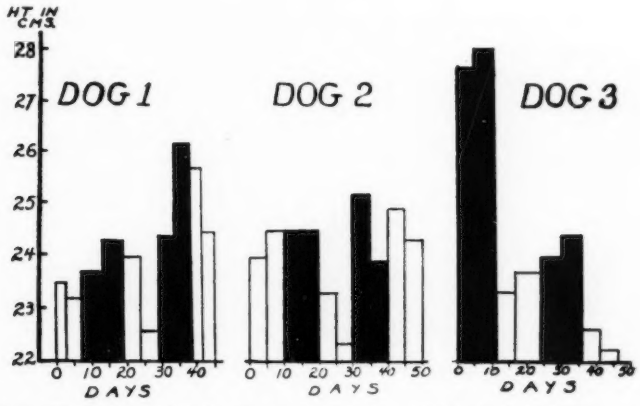

Fig. 4. The average height (in centimeters) to which the left hind paw was lifted in response to the unconditioned stimulus, multiple induction shocks. Black and white areas as in figure 1.

Discussion. Our results are in agreement with those obtained by Zawadowsky, Sacharow, and Slotow (1929), who fed their dogs 0.5 to 1.0 gram of thyroid daily, but are not in agreement with those of Crisler *et al.* (1933), whose doses, 2.5 to 3.0 grams, were closer to ours than Zawa-

dowsky's were. Also, like Zawadowsky and unlike Crisler, we found that thyroid administration increases the magnitude of the unconditioned response. Of course, we dealt with a motor response, while the previous observations were made on secretory conditioned reflexes, and, in this connection, it is interesting to note that Zawadowsky, Sacharow, and Slotow (1929) pointed out that while in dogs a decline of the positive conditioned reflexes is usually accompanied by a simultaneous decrease in differentiation, and, on the contrary, a more exact differentiation is accomplished by a strengthening of the positive salivery reflex, in chickens, under the influence of thyroid (motor reflex; Zawadowsky and Rochlina, 1929) an increase in the positive reflex is usually accompanied by a decline in the inhibitory differentiation, and *vice versa*. The authors presented alternative explanations for these differences: either they are due to the fact that with the dogs a salivary reflex was used, while with the chickens the reflex was motor, or the causes are deeper and are to be found in the predecessors of the nervous systems of these two classes of animals. As our data are similar to those obtained with the salivary reflex in the dog, rather than to the effects on the motor response in the chicken, the second explanation seems to be indicated. Thyroid appears to cause different effects on conditioned reflexes in the dog and in the chicken.

It may be added, in conclusion, that Pavlov and his co-workers consider that anything that stimulates the central nervous system (like caffein) leads to poorer differentiation, while depressing drugs (like NaBr) produce a temporary improvement in differentiation. Chronic administration of thyroid thus resembles the acute effects observed by the Pavlov school after the administration of bromides.

SUMMARY

In five dogs trained to distinguish a metronome frequency of 100 beats per minute from a higher one, by "reinforcing" the former and not the latter, the difference between the positive and negative frequencies was gradually narrowed down to a point where differentiation was no longer perfect. Under these conditions the influence of thyroid upon the differentiating ability was studied by alternating two-week periods of daily oral administration of thyroid in 2.0 gram doses with two-week control periods. There was improvement in the percentage of correct responses to the positive and negative conditioned stimuli during thyroid periods, and a slump in the subsequent control periods. There was also an increase in magnitude of conditioned as well as unconditioned responses during thyroid periods.

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INDIVIDUALITY OF BREATHING[1, 2]

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Present data seem to indicate that chemical changes occurring in the body may produce modifications in breathing in several ways: by direct chemical stimulation of the "respiratory center" (cyanide—Owen and Gesell, 1931) (CO_2—Schmitt, 1932) (sulfide and cyanide—Winder and Winder, 1933); by reflex action exerted through the carotid body and aortic nerve endings (Heymans and Heymans, 1927); and by chemical modification of respiratory reflexes (Gesell and Moyer, 1935b). While it is true that chemical influences not infrequently change the relative degree of costal and abdominal breathing (Gesell and Moyer, 1935a) in addition to the usual changes in rate and depth of breathing (Gesell and Moyer, 1935c) other influences such as those transmitted through the proprioceptive system may be at work controlling the finer integration of the respiratory act. It is this phase of the subject dealing with the selective guidance of the respiratory muscles that we hope to amplify in our studies on the nervous control of breathing.

The present study deals with the participation of individual respiratory muscles in eupnea, a subject presenting little agreement. Howell (1933) summarizes very well the present views on one phase of the subject

> In ordinary quiet breathing the expiration seems entirely passive, but some authors state that in all cases it is accompanied by a contraction of expiratory muscles.

Regarding the specific function of respiratory muscles argument centers mostly around the intercostals. Says Luciani (1911)

> The function of the intercostal muscles, external as well as internal, has, however, been a subject of endless controversy, beginning with the lively polemic between Haller and Hamberger, and lasting into our own day. The most varying and opposite points of view have found strong supporters. Setting aside the opinion of Galen, etc., etc.; there remain four other aspects of the question, which are defended with conflicting arguments by distinguished physiologists, and are set forth in modern text-books:—(a) Both external and internal intercostal muscles are inspira-

[1] Reported in part before the Federation of Biological Societies. Proceedings of The American Physiological Society, This Journal **105**: 37, 1933.

[2] This study was assisted by grants from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry, and the Rockefeller Foundation.

tory (Borelli, Senac, Boerhaave, Winslow, Haller, Cuvier, Duchenne). (b) Both kinds of muscles are expiratory (Vesalius, Diemer-Brock, Sabatier, Beau and Maissiat, Longet). (c) The external intercostals are inspiratory, the internal are expiratory, with the exception of the intercartilaginous portions (Spigel, Vesling, Bayle, and Hamberger, Hutchinson, A. Fick, Martin, and Hartwell).[3] (d) The intercostals are of no great importance in regard to the movements of the ribs; they serve rather to regulate tension in the intercostal spaces, and to reinforce them during inspiration, impeding their retraction by the increased negative intrathoracic pressure (Henle, Meissner, Brucke, von Ebner, Landois).

In our opinion much diversity of conclusions has arisen from inadequacy of methods. Inferences from anatomical relations of muscles, such as points of origin and insertion; direct visual inspection; palpation; and mechanical registration of contraction are dangerous procedures, all susceptible of yielding misinformation in the highly complex respiratory act. Registration of action potentials of muscles in their normal position appears to remove many of the fatal pitfalls of earlier methods and offer the best opportunity of reliable results.

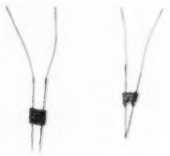

Fig. 1. Floating bipolar electrodes of wide and close approximation. Insulated needles, bare at the tips mounted on balsa wood chips. The wires are phosphor bronze.

Methods. Dogs, anesthetized with morphine and urethane, were fixed to the dog board in the standard supine position with the hind legs and head extended and with the fore legs brought to the side of the back. They were tracheotomized and connected with rebreathing tanks. By deflecting the skin and consecutive layers of muscles the respiratory muscles were sufficiently exposed for the application of floating electrodes and the study of muscle action potentials. The electrodes are pictured in figure 1. They are readily aligned in the direction of the muscle fibers and the tension of the wire is adjusted to permit the electrodes to move freely with the muscle, at the same time maintaining uniform electrical contact. The action potentials are amplified[4] and photographically recorded by a General Electric Multiple Oscillograph.

Tidal air, intratracheal pressure, costal and abdominal respiratory movements, mean blood pressure and heart rate were registered with little inconvenience by substituting mechanical elements for galvanometers in the multiple oscillograph. The mechanical elements consisted of high frequency levers, operating against spring tension, with horizontally moving lever arms attached at right angles to vertically mounted axles. Standard

[3] To these references should be added more recent works. Hough (1893), Bergendal and Bergman (1896), du Bois-Reymond (1896), and Bronk and Ferguson (1935).

[4] The amplifiers were constructed by Mr. F. Schumann of the Electrical Engineering Department.

galvanometer mirrors mounted on the axles served to record respiratory and circulatory events. Tidal air was recorded with the aid of a Hutchinson spirometer, attached to a reducing pulley, which is mounted directly back of and attached, by fine thread, to the tidal air mechanical element. Intratracheal pressure changes were recorded with the aid of an air tambour stretched with a thin rubber membrane capable of recording very small

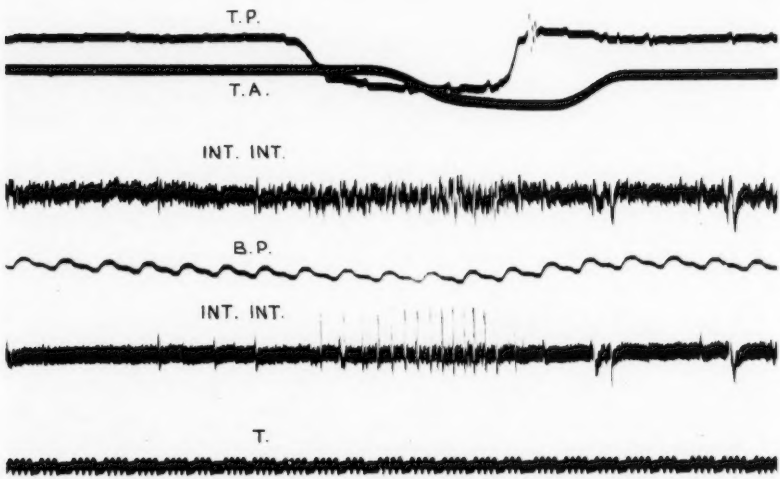

Fig. 2. Sample record of respiratory phenomena obtained with General Electric Multiple Oscillograph, showing inspiratory action potentials of the internal intercostal muscle. The records from below upwards are T. time in 0.04 second, Int. Int., discrete action potentials of internal intercostal muscle with closely approximated electrodes. B.P., blood pressure and pulse rate. Int. Int., indiscrete action potentials obtained from a neighboring portion of the internal intercostal muscle with the electrodes more widely separated. T.A., tidal air as recorded with a Hutchinson spirometer, downstroke indicating the beginning of inspiration and upstroke the beginning of expiration. T.P., tracheal pressure down and up stroke representing the beginning of inspiration and expiration respectively. The T.P. record indicates time relations more accurately due to inertia of spirometer system.

changes in pressure incident to the movement of air. The sensitivity of the manometer was controlled by an adjustable flat spring, such as is used in the Hürthle spring manometer, opposing the movements of the membrane. This protection to the membrane was quite essential with great changes in intratracheal pressure accompanying hyperpnea or inspiratory, expiratory and simple mechanical asphyxia.

Blood pressure and heart rate were recorded with the aid of a membrane manometer connected with the carotid artery and mounted behind the blood pressure mechanical unit. To prevent electrical pick-up of stray signals by a long column of citrate solution, the blood was first led into a gold beater's skin mounted within a glass container filled with and connected by kerosene with the manometer. The manometer was filled with water.

All recorders to the rear of the oscillograph were mounted on vertical rods, pivoted above, on a common horizontal rod. The lower end of each vertical rod was in contact with a threaded control extending to the front of the oscillograph, below the viewing screen, to permit convenient adjustments of the recording light spots. A sample record of the type of results obtainable with our procedure is seen in figure 2.

When the electrodes are nicely approximated, with the ends almost touching, the recorded potentials are likely to be discrete, and of an orderly sequence, easily counted and measured for amplitude, as seen in the lower record. On the other hand, if the electrodes are widely separated the pick-up is no longer localized and the potentials are likely to be more numerous, out of phase, or indiscrete, and lacking orderly sequence and amplitude, as in the upper electrical record.

Results. Among the muscles studied were the M. sternocleidomastoideus, M. scalenius anterior, M. scalenius medius, and M. scalenius posterior, M. serratus anterior and M. serratus posterior, M. pectoralis major and M. pectoralis minor, M. transversus costarum, Mm. intercostales interni, Mm. intercostales externi, M. iliocostalis, Mm. levatores costarum, M. diaphragma, M. triangularis sterni, M. abdominis cutaneous, M. rectus abdominis, M. obliquus externus abdominis, M. obliquus internus abdominis, and M. transversus abdominis. Data were obtained from twenty seven experiments, fifteen of which were performed primarily for other purposes. The procedure and findings in these were consequently less thorough and complete than in the final twelve experiments.

The M. sternocleidomastoideus, M. serratus anterior and M. serratus posterior, and M. pectoralis major and M. pectoralis minor were always inactive. In a very small number of experiments M. scalenius anterior, M. scalenius medius, and M. scalenius posterior contracted during inspiration. The intercartilaginous portions of the Mm. intercostales interni were always active. The Mm. levatores costarum were sometimes resting and sometimes contracting during inspiration. The remaining muscles were either active or inactive varying with the animal or with the conditions as amplified below.

Experimental findings on the function of the Mm. intercostales externi and interni are presented in figures 3 and 4 and table 1. The left hand figures show the activity of the Mm. intercostales externi in eight dogs. The right hand figures show the activity of the corresponding Mm. inter-

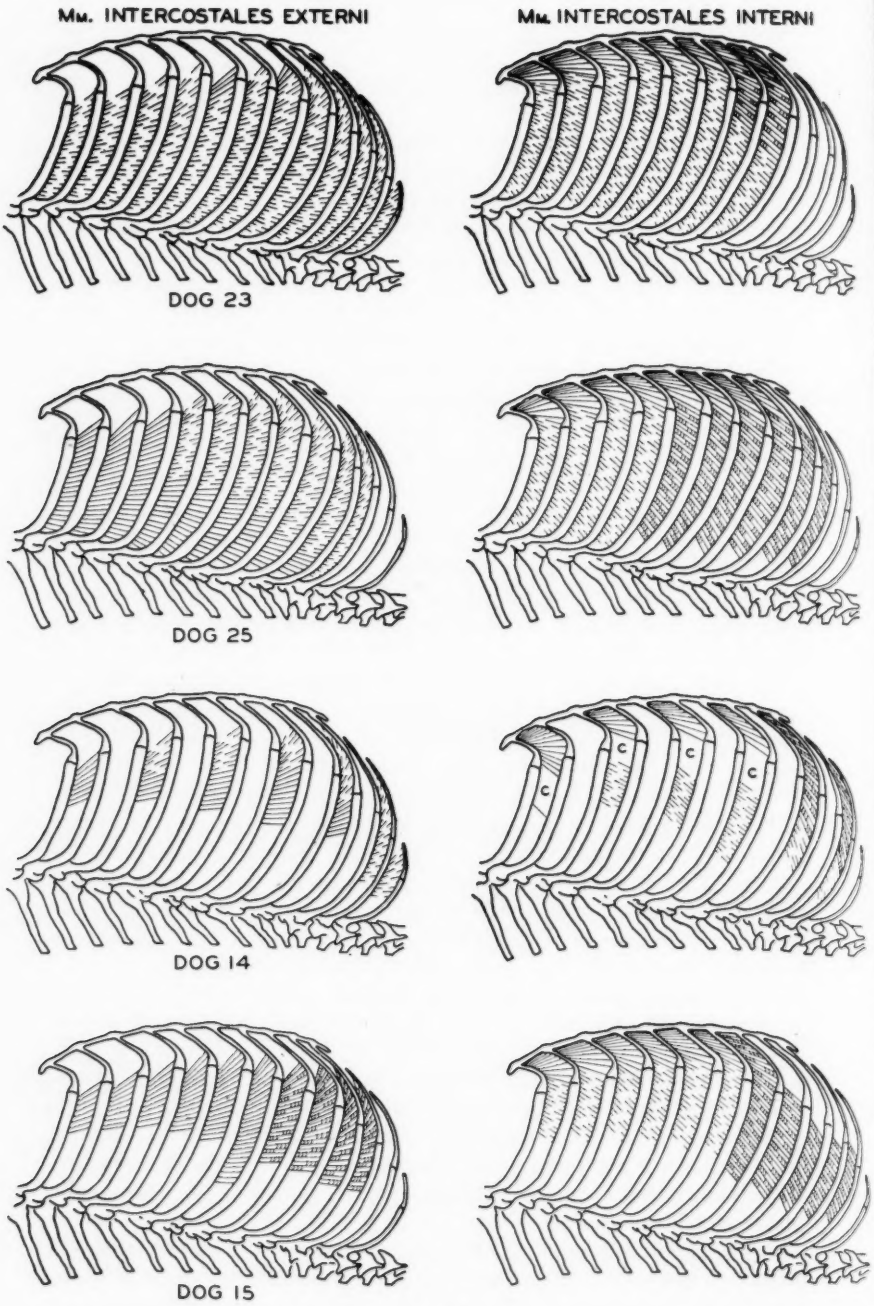

Fig. 3

Figs. 3 and 4. Respiratory patterns of the Mm. intercostales externi and interni of eight dogs during eupnea. Continuous lines indicate activity during inspiration, continuous lines joined by three bars indicate activity during expiration. Broken lines indicate inactivity. Open spaces indicate unexplored regions excepting where labelled C. (continuous activity throughout the respiratory cycle).

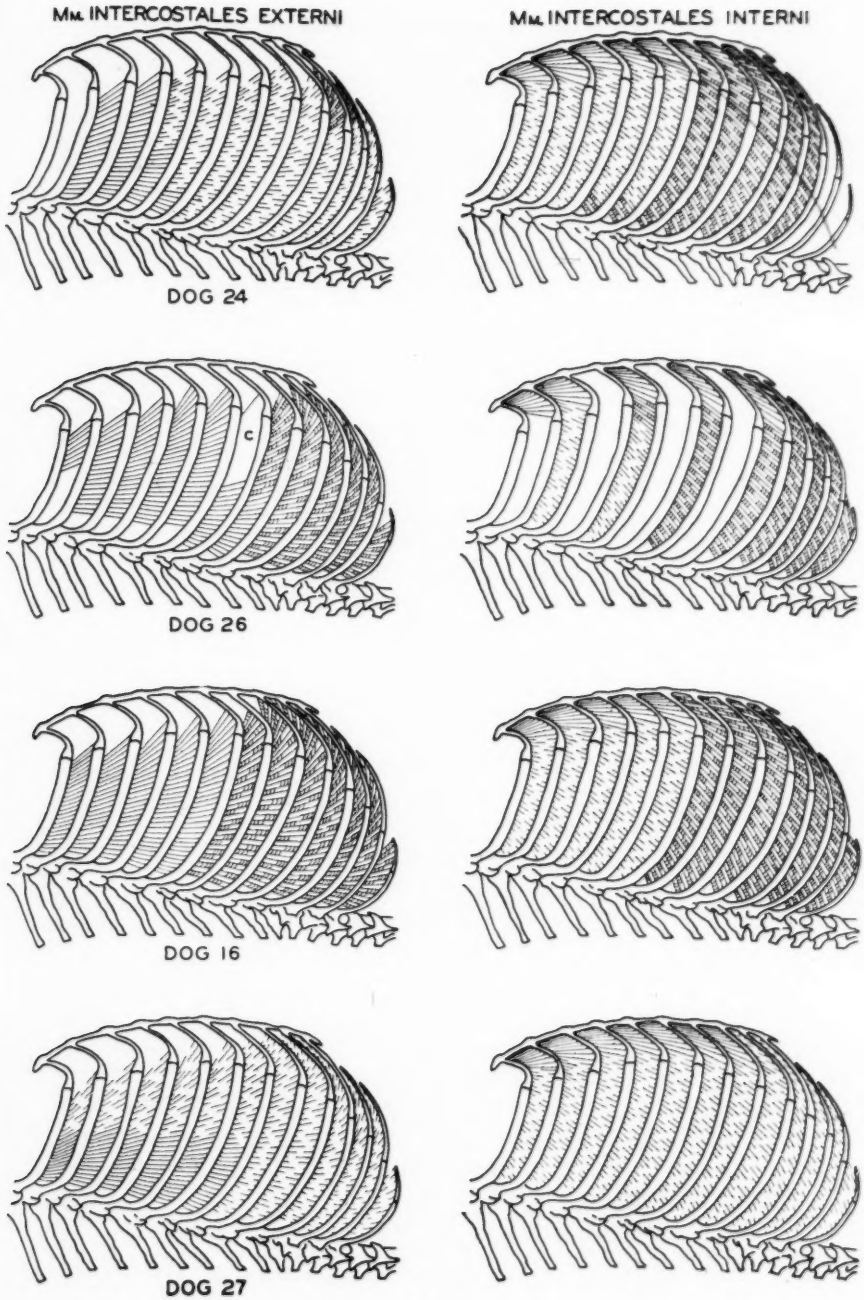
MM. INTERCOSTALES EXTERNI
MM. INTERCOSTALES INTERNI
DOG 24
c
DOG 26
DOG 16
DOG 27

Fig. 4

TABLE 1

Incidence of activity of expiratory muscles and degree of passiveness of expiration

T.S.—M. triangularis sterni, T.C.—M. transcostar. E.A.O.—M. obliquus externus abdominis. I.A.O.—M. obliquus internus abdominis. T.A.—M. transversus abdominis. R.A.—M. rectus abdominis. E.I.—Mm. intercostales externi. I.I.—Mm. intercostales interni.

The lower line gives the percentage incidence of activity of individual muscles during expiration. E indicates contraction during expiration. EC indicates continuous activity but greater during expiration. Blank spaces indicate inactivity.

EXPERIMENT	PASSIVENESS OF EXPIRATION	T.S.	T.C.	E.A.O.	I.A.O.	T.A.	R.A.	E.I.	I.I.
	per cent								
1			0	E					
2			0	E					
3				E	E	E	EC		
4			E	E	E	E			
5			E	E	E	E			
6			E	0	0	0	C		
7			E	EC	EC	0	EC		
8			E	EC	EC				
9			E	E	E		0		
10			0	0		E	0		
11				E			0		
12									
13				E			EC		
14	75.0	E	0	0	0	0	0	0	E
15	12.5	E	E	E	E	E	0	E	E
16		0	E					E	E
17		E		0			0		
18	100	0	0	0	0	0	0	0	0
19			E	E	E	E	0		
20	62	0	0	0	0	E	0	E	E
21	25	E	E	E	E	0	E	0	E
22			E	E	E	E	0	0	0
23	50	0	E	E	0	E+++	0	0	E
24	25	E	0	E	E	E	0	E	E
25	12.5	E	E	E+	E++	E+++	E	0	E
26	12.5	E	E	E++	EC	0	E	E	E
27	100	0	0	0	0	0	0	0	0
		7/12 = 58%	14/22 = 64%	18/25 = 72%	13/19 = 68%	11/18 = 61%	6/20 = 30%	5/15 = 45%	8/11 = 73%

costales interni. The direction of the lines indicates approximately the direction of the muscle fibers. Continuous lines indicate activity during inspiration, deeper shading indicates greater activity. Continuous lines connected at right angles with groups of 3 bars indicate activity during expiration. Broken lines indicate inactivity during both phases of breathing. Open spaces indicate no observations unless specifically labelled to the contrary, e.g., C indicates continuous activity during inspiration and expiration. A glance at the schematic drawing shows the great variability of results which suggests at least one reason for prevailing diversity of opinion.

The potentials of the intercartilaginous portion of the Mm. intercostales interni were strong and gave every indication of the importance of these muscles in breathing. Moving the electrodes laterally from the mesial borders of the muscles, where they are thickest and contracting most actively one clearly hears the volume of the potentials diminish and finally a rather uniformly placed narrow strip is reached where the potentials abruptly cease as in dog 23, interspaces 1–5, and dog 25, interspaces 1–4, or where they suddenly change from the inspiratory to the expiratory type as in dog 24, interspaces 6 and 7 and dog 25, interspaces 5–8.

The inspiratory and expiratory function of these two anatomical divisions of the Mm. intercostales interni was studied and definitely established with the use of mechanical methods by Hough (1893). To quote "That this portion of the internal intercostal muscle should have exactly the opposite function from and an accurately alternating contraction with the interosseus portion, seemed so improbable that this research was started for the purpose of definitely ascertaining the fact." But another fact, equally puzzling, and to our knowledge not described by others, is that the interchondral portions of the Mm. intercostales interni are not always solely inspiratory in function. Only through the first five intercostal spaces was activity invariably in phase with inspiration. From the sixth space on, the muscles were likely to reverse their function and contract alternately with the diaphragm: for example, in dog 16 they contracted during expiration from the sixth to twelfth interspace inclusive. The entire muscle in these spaces contracted during expiration and there was no change in activity or no transition in function to be noted in passing from the chondral to the osseous portion. In dogs 14, 15, and 26 the expiratory function appeared in and caudal to the seventh, seventh and ninth interchondral spaces respectively. In dog 24, about one-half of the sixth interchondral muscle contracted during inspiration and the other half during expiration. In the seventh and eighth interchondral spaces, most of the muscle was expiratory while in the ninth and further caudal spaces, the interchondral muscle was entirely expiratory.

Regarding the interosseus portion of the Mm. intercostales interni,

electrical exploration shows that they are not invariably active during quiet expiration. Inactivity over large areas is common. Scanning the Mm. intercostales interni charts shows that when expiratory activity occurs it is most marked in the caudal half of the chest and that inactivity is most common in the anterior half. We have no observation showing expiratory contraction of all interosseus portions of the Mm. intercostales interni during eupnea but we have two observations of complete inactivity of this entire group. Intravenous injections of sodium sulphide show that active participation of these muscles in the respiratory act can be extended by hyperpnea.

The Mm. intercostales externi are generally considered as inspiratory. Our experiments showed that they were expiratory as well. Inspiratory activity during eupnea was confined mostly to the anterior half or two-thirds of the chest and the dorsal aspect, approximately from the insertion of the M. serratus anterior to the back. When both activities were present in one animal expiratory contractions were limited to the posterior portion of the chest. We have no observation in which all of the Mm. intercostales externi are active either as inspiratory or expiratory. The whole group was active in 3 dogs 15, 26 and 16 where the function was divided. On the other hand, there was one dog in which all Mm. intercostales externi were inactive and two others which, so far as mechanical effects were concerned, could be counted as inactive. Only scattering fibers at the mesial edge of these muscle sheets near the chondral junction contracted with the diaphragm.

Our results on the Mm. intercostales interni and Mm. intercostales externi differ in some respects from the conclusions of Hoover (1922), who finds that "There is a high threshold for their (Mm. intercostales externi) use in expiration, and this is passed only when they are activated synergically with the abdominal muscles. The internal are employed in expiration in hyperpnea, and have a threshold for expiratory use that is lower than that for the external intercostals." Since both Mm. intercostales externi and interni were active during eupnea in our experiments, we must conclude that the threshold for their expiratory use was low. In agreement with Hoover our results indicate that the threshold for expiratory use is somewhat lower in the Mm. intercostales interni.

The question of the passive nature of expiration requires a study of additional expiratory muscles—the M. triangularis sterni, the M. transcostar, and the abdominal muscles. As proved by Hough (1893) the M. triangularis sterni contracts alternately with the diaphragm and is, therefore, expiratory. Action potentials definitely confirm these findings. The M. transcostar (Ellenberger and Baum, 1891) is described as an inspiratory muscle. Granting that the sternum moves cephalad in the classical direction (which it never did in the present experiments, always

moving caudad during inspiration), its points of attachment might indicate a cephalad pull on the sternum thereby assisting inspiration. On the other hand through its connection with the sheath of the M. rectus abdominis it might assist compression of the chest and, therefore, contract during expiration. The difficulty as Martin and Hartwell (1878), Hough (1893) and others have pointed out from time to time is the differentiation of the point of origin from the point of insertion of respiratory muscles and the danger of inferences based on anatomical structure alone. Action potentials show that the M. transcostar when active contracted during expiration.

During excessive breathing the abdominal muscles are admittedly expiratory in function. Only during eupnea is the extent and incidence of contractions debated. Luciani (1877) states that in dogs expiration under normal conditions is always active owing to the intervention of the abdominal muscles. Inspection of table 1, however, shows that under the conditions described for our experiments such results are not invariable. In dogs 18 and 27 all expiratory muscles were inactive. From this condition of complete passiveness of expiration there were many varieties of active expiration as illustrated in table 1. The activity of eight pairs or groups of expiratory muscles is indicated, E standing for activity during the expiratory phase, EC indicating expiratory contractions superimposed on a continuous contraction, 0 indicating inactivity and blank spaces indicating no observation. The table yields four conclusions.

First, the incidence of active expiratory contraction varied with the muscle. (In a total of 24 experiments the M. triangularis sterni was active in 58 per cent of the animals, M. transcostar 64 per cent, M. obliquus externus abdominis 72 per cent, M. obliquus internus abdominis 68 per cent, M. transversus abdominis 61 per cent, M. rectus abdominis 30 per cent, Mm. intercostales externi 45 per cent and Mm. intercostales interni 73 per cent.)

Second, the degree of passiveness of expiration varied from dog to dog. (As crudely analyzed in column 1, giving a value of 12.5 per cent for each inactive muscle or muscle group—dogs 18 and 27 showed 100 per cent passiveness; dog 14, 75 per cent; dog 20, 62.5 per cent; dog 23, 50 per cent; dogs 21 and 24, 25 per cent, and dogs 15, 24, and 26, 12.5 per cent. No animal showed simultaneous participation of the eight sets of muscles in eupneic expiration.)

Third, the muscle pattern of active expiration differed in every animal with the exception of dogs 18 and 27 where expiration was completely passive but in these two dogs the inspiratory patterns were different. We can, therefore, say that the muscle pattern of breathing in all of our dogs differed from one animal to another.

Fourth, the intensity patterns differed from dog to dog. (In dog 25 the

M. obliquus externus abdominis contracted very weakly, the M. obliquus internus abdominis more strongly and the M. transversus abdominis still more energetically. In dog 23 the intensity arrangement was quite different. The M. transversus abdominis again contracted most vigorously, the M. obliquus internus abdominis was inactive and the M. obliquus externus abdominis was active only in a narrow band passing over the eighth rib. In dog 22 only the upper halves of the M. obliquus externus abdominis and M. obliquus internus abdominis contracted. In dog 25 only narrow bands passing over the lower ribs were active.)

Adding to these respiratory patterns the pattern of muscle sequence, to be described in a later paper, we are impressed with endless possibilities in variability or individuality of the respiratory act along several fundamental lines. Admittedly these differences might originate from variations in central organization, a subject about which we know very little. Conceivably, too, these variations might have their source in reflexes initiated at the periphery, controlling in a selective way the activity of the respiratory muscles. If so, an explanation of the integration of the respiratory act will hinge upon our knowledge of the mechanics of breathing and the mechanics of the distortion of the proprioceptive endings residing in the mechanical system.

SUMMARY AND CONCLUSIONS

The objective of our experiments is to determine the incidence and degree of activity of the respiratory muscles during eupnea as a foundation for further experiments on the nervous control and integration of the respiratory act.

A brief review of the literature on the activity of respiratory muscles shows a great diversity of opinion and a need for further study.

Systematic mapping of inspiratory and expiratory potentials of respiratory muscles was the procedure employed. This method offers many advantages over those of mechanical registration and of inferences based on anatomical considerations.

Methods of simultaneous registration of action potentials and respiratory and circulatory events are described.

Results from a series of twenty-seven experiments show great variations in participation of respiratory muscles in the act of breathing. This may account for much of the diversity of opinion on the function of these muscles.

The interchondral portion of the Mm. intercostales interni and the M. diaphragma invariably participated in the act of breathing. Participation of the interosseus portion of the Mm. intercostales interni, Mm. intercostales externi, Mm. levatores costarum, M. transcostar, M. triangularis sterni, M. obliquus externus abdominis and M. obliquus internus abdom-

inis, M. transversus abdominis and M. rectus abdominis varied with the individual animal.

The interchondral portion of the Mm. intercostales interni contrary to current opinion was not exclusively an inspiratory muscle. From the sixth, seventh, eighth, or ninth interspaces it contracted, in scattering cases, during the phase of expiration.

The interosseus portion of the Mm. intercostales interni varied greatly in activity. Extensive non-activity was common. Expiratory activity occurred mainly in the posterior half of the chest.

The Mm. intercostales externi varied in function and activity. Inspiratory contractions were confined mainly to the anterior half and dorsal aspects of the chest. Expiratory activity was confined to the posterior half. Simultaneous inspiratory and expiratory activity in these respective areas was not uncommon.

The remaining muscles (M. obliquus externus abdominis, M. obliquus internus abdominis, M. transversus abdominis, M. triangularis sterni and M. transcostar) all expiratory in function, varied in incidence and degree of activity.

The M. cutaneous abdominis frequently functioned as an expiratory muscle.

Combined analysis of the results on inspiratory and expiratory muscles indicates that every individual possesses its individual respiratory pattern of breathing. Individuality of breathing is a resultant of varying combinations of varying muscle patterns, of varying contraction intensity patterns, and varying muscle sequence patterns.

In two dogs muscular activity during the expiratory phase of breathing was entirely missing. It must, therefore, be concluded that expiration may be a purely passive phenomenon.

In the remaining twenty-five dogs varying degrees of active expiration were encountered.

The significance of the diversity of respiratory patterns is briefly discussed.

I wish to acknowledge the valuable help of Dr. F. White in this research.

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VASOMOTOR RESPONSES OF THE MUCOSA OF THE UPPER RESPIRATORY TRACT TO THERMAL STIMULI

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Although several studies have been reported concerning vasomotor responses of the respiratory tract mucosa to thermal changes on the surface of the body, several problems are still unsolved. Hill and Muecke (1) were the first to show that chilling of the cutaneous surface was followed not as was commonly supposed by vasodilatation of the mucous membrane of the nose and throat but by an ischemia. Conversely, in a warm atmosphere the mucous membrane of the nose became swollen and hyperemic. Later studies by Hill (2) and the New York State Commission on Ventilation (3) yielded confirmation. Thermoelectric studies of the temperature of the mucous membrane as an index of vascular changes have been made by Mudd and his co-workers (4), Azzi (5) and Winslow and Greenburg (6). Undritz and Sassossow (7) made similar observations in the dog and rabbit. Nedzel and Arnold (8) extended these studies to observations on the temperature of the mucosa of the trachea and bronchi in dogs. They found reflex lowering of the mucosal temperature of the whole tracheo-bronchial tree on chilling the skin.

There is general agreement among observers that rapid cooling of most cutaneous surfaces produces a reflex vasoconstriction leading to lowered mucous membrane temperature in the whole upper respiratory tract. There is still uncertainty regarding the lower limits of area exposed to cold necessary to produce such effects. The importance of pain sensations in connection with extreme cold has moreover not been investigated. Nor have repeated observations been made on the type of response elicited in the same subject over several months' time. The latter is of importance to an understanding of the pathological physiology of the respiratory mucosa. Our experiments were conducted to study these problems.

METHODS. One hundred and ninety-eight experiments, distributed as follows, were conducted: 81 general experiments on one normal man, 54 experiments on 9 normal men, 32 experiments on 15 frequent head cold cases (infectious), 31 on 15 frequent head cold cases or hyperesthesias (non-infectious). These were conducted in a laboratory room with an

air temperature ranging between 21 and 26°C., in which drafts were minimized. They were performed on the same subject daily at the same time, usually in the morning, 1½ hours after breakfast.

At the beginning of an experiment the subject entered the room and disrobed at once and remained at ease either sitting or reclining for 30 minutes. Records were made of the following: 1, subject's physical and mental status during the preceding 24 hours, i.e., his usual routine, or any exceptional physical and emotional activities; 2, pulse and respiratory rates, and mouth temperature; 3, the condition of his nasal and oral mucosa.

Cold air, cold sprays, cold baths, and aluminum cups, varying in diameter from 4 to 19 cm., filled with chipped ice were used as cold stimuli in different experiments. The cold air was provided by opening windows and directing an electric fan to the back. Cold sprays and cold baths ranged in temperature from 3 to 19°C. The ice-filled cups gave a surface skin temperature of 12.6°C. within a minute. Hot sprays, hot baths and aluminum cups filled with hot water were used as heat stimuli. The hot water ranged from 42.5° to 43.5°C. Cups were applied to the upper and lower dorsal and lumbo-sacral regions of the back, to the upper and lower anterior chest, to the upper and lower abdomen, and to the feet.

The temperature of the nasal mucosa was measured with a thermopile applied, with constant pressure, to the mucosa of the nose and the E.M.F. measured through a potentiometer. A three element thermopile made of German silver wire no. 30 and of copper wire no. 25 was used. Three lengths of the German silver wire, 1 m. long, were soldered alternately to three pieces of copper wire of the same length. Pure silver solder was used. The wires were then folded together to make a single bundle of 6 strands. Mountings of stiff, narrow bakelite were provided for both the known and unknown temperature ends of the thermopile system. The wires were placed in grooves cut into the bakelite, 2 wires in a groove, and the electrode was then made waterproof with celluloid cement dissolved in acetone. The constant temperature thermocouple was fitted into a rubber stopper of a thermos bottle filled with shaved ice and carefully packed to avoid air spaces. The unknown temperature end of this thermopile was attached to a specially constructed head gear and electrode holder to which was fitted a micrometer. The thermopile terminals were thereby fixed upon the site to be studied so that they would remain unchanged in their position, under constant and moderate pressure, and covering such a small area as not to interfere with the rise and fall of temperature in the surface under them in response to changing vasomotor conditions.

Results. I. *Normal reactions.* a. *Cold air stimulus.* Uncovering the trunk and exposing it to cold air caused a decided drop in mucous membrane temperature; wrapping the trunk produced a rise in temperature.

b. *Cold and hot water stimuli.* Cold water applied to the back produced a marked drop in the mucous membrane temperature, hot water a marked rise. See figure 1. Cold and hot baths, because of the greater amount of skin surface stimulated, produced more marked changes in the mucous membrane temperature.

c. Cold and hot stimuli by means of aluminum cups applied to the upper back between the 3rd to the 7th dorsals, the area found to be, after much experimentation, the most sensitive, produced the following: 1, a 4 cm. ice cup was the smallest cold stimulus to give a reflex lowering of the temperature of the nasal mucosa; 2, the normal response to a short circumscribed cold stimulus (2 min.) was first a lowering of the mucous membrane temperature, then a rise and a gradual return to the initial temperature. 3. The normal response to a continuous circumscribed cold stimulus (10

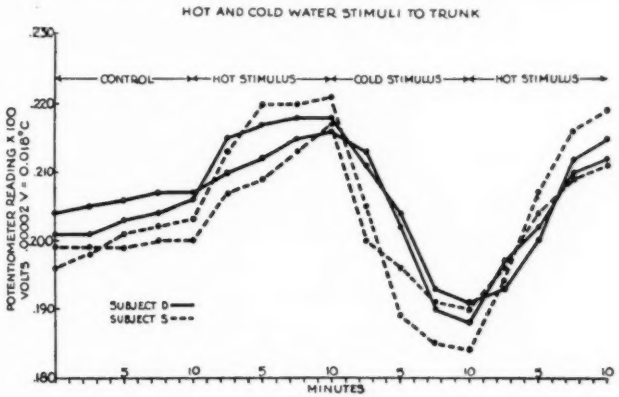

Fig. 1

min.) to one cutaneous area or to numerous cutaneous areas applied in regular succession, i.e., when the reaction was completed in one area the stimulus was immediately removed to another, was first a lowering of the mucous membrane temperature and then a gradual rise, stopping, in the majority of the experiments from reaching the initial temperature by 0.18 to 0.36°C. The areas stimulated in this succession were from the upper and lower dorsals to the lumbo-sacral regions of the back, to the upper and lower anterior chest, to the upper and lower abdomen, and last to the feet. The wearing off of the effect when a cold stimulus is maintained indicates end organ fatigue or adaptation. It is proven to be of peripheral and not central origin by the fact that application of cold to a second area after a first effect wears off shows a new response comparable to the initial reflex. See figure 2. 4. The effect of a gradual cold stimulus. When a cold stimulus was applied so that the temperature in the cup was

gradually reduced from 29° to 9°C. there was no appreciable change in the nasal mucous temperature until the temperature within the cup reached 9°C. and then the drop of the nasal temperature was only 0.36°C., considerably less than if the cold stimulus were started with 9°C.

d. A cold stimulus applied to an anesthetized cutaneous area. After it was found that cold stimuli applied to cutaneous areas by means of iced

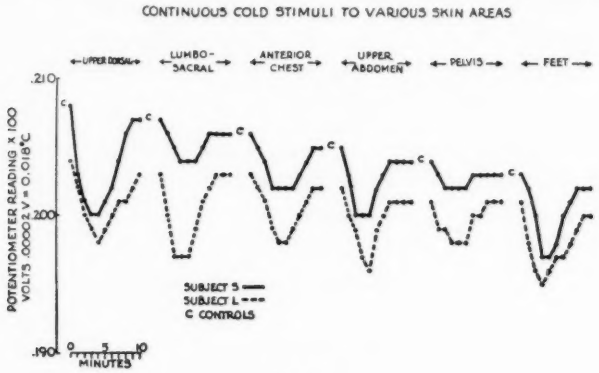

Fig. 2

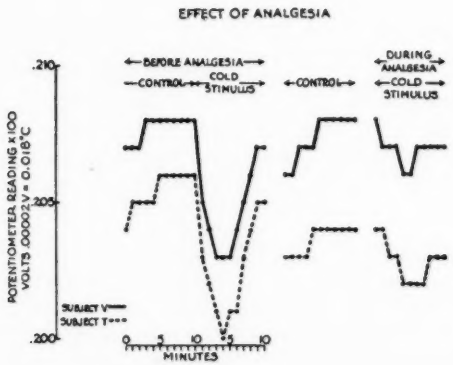

Fig. 3

aluminum cups produced both cold and pain stimuli, with the larger cups producing a great deal of pain for the first few minutes, and since thermal impulses take a similar course to pain, we believed that the reflex-vasomotor lowering of the nasal mucous temperature to a cold stimulus might be enhanced by pain stimuli, we therefore conducted experiments under analgesia. The skin area of the upper back between the 3rd and the 10th dorsals greater than the surface area of the 12 cm. ice cup was made an-

esthetic to touch and pain by injecting subcutaneously a butyn epinephrine solution $\frac{3}{4}$ per cent. After pain sensations were absent cold was still felt although with lowered sensitivity. Two subjects were used for this experiment. The results were that the cold stimulus applied to the analgesic area still produced a reflex lowering of the mucous membrane temperature. This appears to prove that pain impulses are not the determining factor in producing the reflex vaso-constriction. See figure 3. The decreased effect is to be explained in all likelihood on the grounds of partial anesthesia to cold.

II. *Irregular reactions to cold stimuli in normals.* The 9 normal subjects studied were stimulated daily. Occasionally on Mondays following a week-end of unusual emotional activity, the normal gave irregular reactions, although for many previous and successive days his reactions followed the normal pattern. These irregular vasomotor responses to a cold

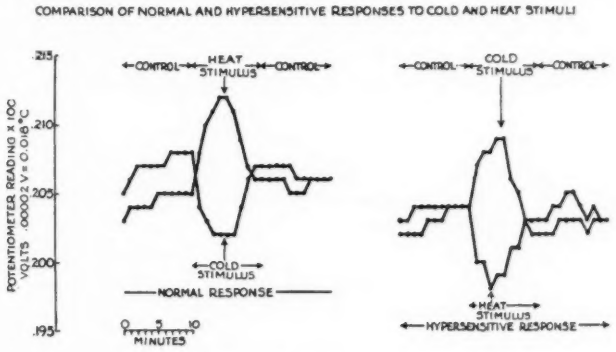

Fig. 4

stimulus were as follows: *a*, a very much delayed reaction to a given cold stimulus; *b*, no response whatever to a cold stimulus; *c*, in two instances the reaction was reversed—a cold stimulus produced a rise and a heat stimulus a fall in the mucous membrane temperature. The latter two instances occurred in different men following excessive emotional strain.

III. *Abnormal reactions.* a. *Head colds* (*infectious*). During the months of April and December several of our subjects, normal men, developed head colds. These were studied. Just prior to, during and following a head cold, there is a period of vasomotor suspense, i.e., a cold stimulus applied to the cutaneous surface produces no vasomotor response in the mucous membrane. This suspense of the vasomotor reaction is presumably due to local changes within the mucosa, produced by the toxic products of the infection upon the vasomotor endings.

b. *The frequently repeated head cold* (*infectious*). The vasomotor re-

sponse to a cold stimulus in patients who gave a long history of many head colds with fever was as follows: 1, in the interval between infections the response of these patients to a cold stimulus was very much delayed. Whereas in normals the reaction to a circumscribed cold stimulus was completed in ten minutes, in the infectious cases such a reaction took 20 minutes and longer; 2, very frequently infectious cases when stimulated with cold presented a lowering of the mucosa membrane temperature which remained depressed, sometimes for several hours. Poor vasomotor response of the nasal mucosa to a cutaneous cold stimulus in infectious cases is perhaps responsible for infections. A nasal mucous membrane for a prolonged period in a state of ischemia when present in the environment of an infectious process may become infected because of its lowered resistance during this ischemic state.

c. *The frequently repeated head cold (non-infectious, i.e., nasal hyperesthesia).* The hyperesthesia cases gave erratic vasomotor responses to thermal stimuli. Most frequently a cold stimulus applied to the cutaneous surface produced a reflex rise in the nasal mucous temperature instead of a fall, a heat stimulus produced a fall instead of a rise. See figure 4.

SUMMARY AND CONCLUSIONS

1. The normal response to a short circumscribed cold stimulus is first a lowering of the mucous membrane temperature, then a rise and a gradual return to the initial temperature.

2. The normal response to a continuous circumscribed cold stimulus is first a drop, and then a rise in temperature. The return, however, stops short of the initial temperature by 0.18 to 0.36°C. Cold applied over areas larger than 4 cm. diameter regularly produced reflex mucosal vasoconstriction. Smaller areas of stimulation were not effective.

3. The anesthesia experiments proved that with pain removed, cold stimuli over the anesthetized area still produced a reflex lowering of the mucous membrane temperature indicating that pain does not produce the vasomotor changes in the nasal mucosa.

4. During a head cold the vasomotor response of the nasal mucosa to a cold stimulus applied to the cutaneous surface is altered. The head cold patient of the infectious group usually gives a much delayed vasomotor response to cold stimuli. The head cold patient of the non-infectious group (hyperesthesias) produces reversed reactions to cold stimuli. Cold applied to the cutaneous surface increases the nasal mucous temperature.

Changes in the temperature of the mucosa of the upper respiratory tract were proven in these experiments to be the result of a change in the vasomotor tone in the vessels supplying these membranes. A fall in temperature indicated a vasoconstriction, a rise, a vasodilatation. These

experiments further showed that chilling of the body surface caused a reflex vasoconstriction and ischemia in the upper respiratory mucous membrane. This is contrary to the earlier explanations that such chilling caused a congestion of the mucous membranes.

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THE ABSORPTION OF CYSTINE, METHIONINE AND CYSTEIC ACID FROM INTESTINAL LOOPS OF DOGS[1]

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Several studies of the absorption of various amino acids and their derivatives from the small intestine have appeared in recent years (Wilson, 1930; Sullivan and Hess, 1931; Andrews and Johnston, 1933; Chase and Lewis, 1933). Among various procedures employed, we have found that the use of modified Thiry jejunal loops (Johnston, 1932) offers certain advantages over other methods in that repeated determinations can be made on the same unanesthetized animal and more strictly comparable results thus obtained than if separate animals are used for various determinations. Moreover, the ease with which these loops can be freed from intestinal flora makes possible measurements which are uncomplicated by bacterial decomposition of the substance studied—a particular advantage in the case of the sulfur compounds with which this paper deals. Using dogs with isolated jejunal loops, we have already reported (Andrews and Johnston, 1933) some studies of the relative rates of absorption of cystine and some related sulfur compounds. In the present paper we wish to report similar but more detailed studies applied to the absorption of l-cystine, dl-methionine and l-cysteic acid. The technique employed was identical with that described in the paper cited above with the exception that, as a rule, 6 hour periods of absorption were employed. The unabsorbed residue in the loop was quantitatively washed out and total sulfur determined gravimetrically on aliquots of the same by the Benedict procedure, with precipitation as $BaSO_4$.

We have been able to demonstrate that repeated experiments on one dog, run day after day, appear to produce a fatigue which results in lower absorption values. Far more comparable results have been obtained by allowing at least one day of rest between each experiment and this pro-

[1] An abstract of portions of this paper was presented at the meetings of the American Association for the Advancement of Science held in Pittsburgh, December 1934. An abstract was also published in the Proceedings of the American Society of Biological Chemists, viii, no. 4.

We wish to acknowledge the assistance afforded by a grant from the Faculty Research Committee and also the technical assistance of Mr. A. L. Ellis.

TABLE 1

Absorption of cystine, methionine and cysteic acid from the intestinal loop of a dog

Time of absorption, 6 hours

	CYSTINE ADMINISTERED								
	0.500 gram			1.000 gram			1.875 grams		
	Iso-electric	Na salt		Iso-electric	Na salt		Iso-electric	Na salt	
	Cystine absorbed	Cystine absorbed	Alkali absorbed	Cystine absorbed	Cystine absorbed	Alkali absorbed	Cystine absorbed	Cystine absorbed	Alkali absorbed
	grams	*grams*	*cc. 0.1 N*	*grams*	*grams*	*cc. 0.1 N*	*grams*	*grams*	*cc. 0.1 N*
	0.368	0.333	34.6	0.367	0.406	35.5	0.413	0.403	57.0
	0.337	0.340	37.2	0.358	0.341		0.375	0.439	45.5
	0.351	0.337	32.8	0.358	0.340	41.5	0.415	0.428	52.0
Average.....	0.352	0.337	34.8	0.361	0.362	38.5	0.401	0.423	51.5
Percentage absorption.....	70.4	67.4	83.6	36.1	36.2	46.3	21.4	22.6	33.0

	METHIONINE ADMINISTERED								
	0.621 gram			1.242 grams			2.484 grams		
	Iso-electric	Na salt		Iso-electric	Na salt		Iso-electric	Na salt	
	Methionine absorbed	Methionine absorbed	Alkali absorbed	Methionine absorbed	Methionine absorbed	Alkali absorbed	Methionine absorbed	Methionine absorbed	Alkali absorbed
	grams	*grams*	*cc. 0.1 N*	*grams*	*grams*	*cc. 0.1 N*	*grams*	*grams*	*cc. 0.1 N*
	Complete absorption		26.4	0.998	0.988	27.9	1.54	1.47	57.8
			27.6	0.972	1.08	34.0	1.43	1.50	50.0
Average.....			27.0	0.985	1.034	30.9	1.48	1.48	53.9
Percentage absorption.....			64.8	79.3	83.2	37.2	59.5	59.5	32.4

	CYSTEIC ACID ADMINISTERED								
	0.704 gram			1.408 gram			2.816 grams		
	Iso-electric	Na salt		Iso-electric	Na salt		Iso-electric	Na salt	
		Cysteic acid absorbed	Alkali absorbed		Cysteic acid absorbed	Alkali absorbed		Cysteic acid absorbed	Alkali absorbed
		grams	*cc. 0.1 N*		*grams*	*cc. 0.1 N*		*grams*	*cc. 0.1 N*
		0.556			0.761			1.16	
		0.572			0.762			1.20	
Average.....		0.569			0.762			1.18	
Percentage absorption.....		80.8	80.8		54.1	54.1		42.0	42.0

cedure has accordingly been followed with all of the experiments herein recorded.

It is of interest to note that all of the present studies were made on one animal: the "dog 36" of the paper by Andrews and Johnston (1933). This dog has been used for studies of the absorption of various substances since September 1932. Its present weight is 19 kilos.

The most significant changes we have observed in the properties of the loop over this length of time have been first, in the total sulfur content of the mucus and secretion washed out after a period of time equal to that of the absorption experiments, and second, in the rate of absorption of cystine and cysteic acid. The "intestinal blanks" reported by Andrews and Johnston in 1933 for 4-hour periods averaged 0.034 gram $BaSO_4$. Our present values for 4-hour blanks average 0.072 gram while those for 6-hour blanks average 0.115 gram $BaSO_4$. As previously reported, the successive blank determinations have always given remarkably concordant results. The figures in table 1 of this paper have been corrected for both the reagent blank and the intestinal blank (0.115 gram $BaSO_4$).

During the two years that elapsed between our earlier experiments (Andrews and Johnston, 1933) and those reported in this paper the rates of absorption of cystine and cysteic acid from this loop have markedly decreased. Although the figures in table 1 refer to 6-hour periods of absorption, we have also repeated the determination with cystine at various times for 4-hour periods with 0.500 gram charges of isoelectric cystine as in our earlier work. Whereas the earlier figures show an average of 0.340 gram cystine absorbed in 4 hours, determinations made 2 years later showed values of 0.100, 0.108 and 0.104 gram absorbed under the same conditions. As regards cysteic acid a similar decrease has been observed. In the earlier experiments 0.704 gram cysteic acid was completely absorbed in 4 hours. We now obtain an average absorption in 6 hours of 0.569 gram.

In table 1 are summarized the results of a number of such absorption experiments with cystine, methionine and cysteic acid. All figures in this table refer to 6-hour absorption periods. With each amino acid three levels of administration were used and for each level, the amino acid was administered both in the isoelectric form and as the sodium salt, except in the case of cysteic acid which, because of its highly acidic sulfonic acid group cannot be administered as the free ampholyte without damage to the intestine. Amounts of all three substances containing equivalent amounts of sulfur are compared except in the highest level of administration where the cystine used was that equivalent of 0.500 gram S. In those instances with cystine and methionine in which the amino acid was administered as the sodium salt (the weighed sample + the theoretical equivalent of standard sodium hydroxide) the absorption of the alkali was also determined independently by titration of an aliquot with standard acid using methyl

red as indicator. This brings the titration to sufficiently near the isoelectric point of both cystine and methionine to permit a practically quantitative titration of all of the alkali remaining, irrespective of the amount of amino acid. One gram cystine (or 1.242 gram methionine) requires the equivalent of 83.2 cc. 0.1 N NaOH for complete salt formation. The figures in table 1 represent the amount of the alkali absorbed in terms of cubic centimeters 0.1 N NaOH. Since cysteic acid behaves as a strong acid, any difference between the rate of absorption of the acid and the base combined with it would be at once apparent in the acidity or alkalinity of the material removed from the loop and the excess base or acid could be easily titrated. However, it may be stated here that no such independent absorption of acid and base was observed in the case of cysteic acid. The findings with cystine and methionine in this regard will be discussed below.

Table 1 indicates that increasing the charge of either amino acid increases to a moderate degree the amount absorbed. This increase has been consistently observed in spite of extraordinary precautions to prevent loss of recovered sulfur, either in washing the compound from the loop or in the determination of total sulfur. In the case of cystine, particularly when administered in isoelectric form, washing the loop with dilute alkali as described in our first paper, is necessary. After this washing was considered completed, a further washing with dilute alkali was made and tested with cyanide and nitroprusside. Only when this test was negative was washing considered complete. In all experiments with larger amounts of each amino acid the total amount of recovered material was made up to a definite volume and aliquots were taken for analysis. When cystine was administered as the sodium salt and aliquots were also desired for titration of total base the washings with water were kept separate from those with dilute alkali and sulfur was determined in aliquots of both.

The comparative sterility of the loop was again emphasized by the fact that nitroprusside tests (without cyanide) on the material recovered after administration of either cystine or methionine were uniformly negative. There was thus no evidence of formation of hydrogen sulfide or of any sulfhydryl derivative in the loop.

The comparative slowness with which cystine is absorbed suggested that administration as the disodium salt, which is extremely water soluble as contrasted with isoelectric cystine, might result in more rapid absorption. However, the data in table 1 demonstrate rather surprisingly that introducing the cystine as the salt has no effect whatever on the rate of absorption, nor was any difference found between salt and free ampholyte in the case of methionine. Further experiments with cystine in which the absorption of the free compound and its sodium salt were compared for a shorter period (3 hrs.) also gave identical values for both forms. The figures representing the absorption of the alkali in terms of cubic centimeters

0.1 N NaOH show that the rates of absorption of amino acid and of alkali are quite independent of each other and that the salt, when administered as such is not absorbed as such but as a mixture of two independent substances. This is emphasized by the data obtained for percentage absorption of the separate substances. The figures for alkali are consistently higher than are those for equivalent amounts of cystine but lower than are those for the more rapidly absorbed methionine. These figures would appear to indicate that the different amino acids and the base have their own specific rates of absorption and that this specificity is maintained in spite of salt formation between alkali and amino acid. The more rapid absorption of methionine observed by us agrees with the data reported by Chase and Lewis (1933) who obtained values averaging 53 mgm. per hour per 100 grams body weight with rats as compared with a figure of 30 mgm. for cystine (expressed in the same manner) obtained by Wilson (1930) and substantially confirmed by Sullivan and Hess (1931) when using the Folin procedure. Our own results show an even greater divergence between the rates of absorption of these two compounds.

The data on cysteic acid absorption furnish an interesting contrast to those on the absorption of cystine and methionine. With cysteic acid we find no evidence whatever of the independent absorption of base and amino acid which is indicated in the absorption of the other two substances. This would imply that the possibility of a hydrolytic mechanism may be the deciding feature. Cystine and methionine both have sufficiently weak acidic constants to permit hydrolysis of the sodium salt whereas with the sodium salt of cysteic acid such a process is no more to be expected than with sodium chloride. One can easily conceive that this hydrolysis, where possible at all, is greatly increased by some type of membrane equilibrium.

SUMMARY

1. The relative rates of absorption of l-cystine, dl-methionine and l-cysteic acid have been measured in a dog with an isolated intestinal loop with the result that methionine appears to be absorbed about three times as rapidly as cystine while the cysteic acid is absorbed at a rate intermediate between the other two.

2. The amount of the amino acid absorbed in a given length of time varies somewhat with the amount placed in the loop: the larger the dose the more is absorbed.

3. The amount of the amino acid absorbed is the same, whether administered as the free ampholyte or as the sodium salt.

4. The amount of the base absorbed is sufficiently specific and independent of the amino acid with which it is combined as to suggest that its absorption is an independent process.

5. The ability of the animal used in these studies to absorb cystine and cysteic acid has been shown to have decreased to about one-third during a period of 2 years.

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THE EFFECT OF RADIATION ON THE EXCITABILITY OF SMOOTH MUSCLE

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Although several papers have already been published on the effect of radiation of smooth muscle (Adler, 1920; Harris, 1925; Hill and Azuma, 1926; Kolm and Pick, 1920; and others) up to date the only measure of the effects of the various treatments given was to note the change in the frequency and height of the rhythmical contractions.

TECHNIQUE AND APPARATUS. In the course of this investigation the stomach of the frog, *Rana pipiens*, was employed after the animal was pithed. While previous investigators in this field used the intact organ according to the technique of Magnus (1904) we utilized cross sections of the stomach and the fact that each strip was cut so as to be about 3 mm. in width made it possible to obtain three strips from each stomach. In obtaining these strips great care was exercised as any undue pulling or tension on it, or on any other part of the organ, may easily induce rhythmical contractions. The absence of rhythmical contractions in the normal preparation was necessary as the excitability of the tissue was measured by the response of the strip when stimulated by means of a thyratron chronaximeter (Thorne, 1933). The use of this apparatus made it possible to apply various voltages (range 100 volts to 33 micro volts) for times varying from 3,720 sigma down to 0.00372 sigma. The current (applied through silver electrodes) was reversed after every stimulation. The segments were always kept moist with Ringer's solution which was kept at 20 to 22°C. The above procedure was quite necessary because of the possible temperature effects on protein (Lepeschkin, 1931). For examination the strips were placed on a smooth muscle lever, designed by Mr. B. R. Macmillan, and allowed to stretch (Evans, 1926, and Grutzner, 1904) for about 15 minutes under the slight weight of the aluminum lever. Ringer's solution at 20–22°C. was kept dripping over the strip during the course of the experiment. The contractions were recorded on a kymograph moving at 1.4 cm. per minute.

The visible radiation was furnished by a 500 watt General Electric flood lamp, with reflector, and was placed at 50 cm. from the preparation with

a water cell, 10 cm. in diameter, between the source and preparation while the strip was on the lever. Ultra violet radiations were furnished by a 500 watt Cooper-Hewitt quartz mercury arc at 50 cm.

The normal variation in rheobase and the effect of time, ultra violet and visible radiation on the rheobase of smooth muscle strips. The average rheobase of 52 freshly dissected stomach strips was 1.22 volts. It appeared that the distribution of rheobase values shifts to a slightly higher value after standing for 5 hours or more and after standing for 15 hours the normal distribution curve becomes very distorted.

When the stomachs were dissected and strips were obtained, of necessity one strip was near the cardiac end of the organ, one in the central portion, while the third was from the region of the pylorus. This being the situation, it appeared quite necessary to ascertain whether or not there existed any difference in rheobase of strips composing the various regions of the stomach and in an effort to obtain this information the next series of experiments was undertaken.

Six stomachs were employed and 18 strips were observed. The results of this series of experiments showed a variation of less than 4 per cent among the three different groups and thus it appears that there is no significant difference in the excitability of the strips from the regions which furnished the segments used during the course of this investigation.

Another series of experiments was performed in order to determine the change in rheobase at various intervals after dissection. The findings on this series indicate that segments utilized within 3 hours after dissection, and under the circumstances which prevailed during the course of this investigation, will furnish an accurate index of rheobase value, since during this period the average variation of rheobase value was 4 per cent.

The effect of ultra violet irradiation on smooth muscle preparations was tested in 14 experiments. In all of these an increase in excitability followed irradiation and figure 1 shows 3 typical curves indicating the effect of radiation on the rheobase of the tissue (a decrease in voltage per cent signifies a fall in rheobase and an increase in excitability). It was found that short intense irradiation produced a marked increase in excitability and after irradiation ceased the tissue did not exhibit any recovery towards the initial level of excitability.

Ten experiments were performed utilizing radiation from the visible range and figure 2 shows typical curves exhibiting the effect of visible irradiation. There is a marked increase in excitability during irradiation and a fairly rapid return to the initial level after stopping irradiation. It should be noted that visible irradiation does not produce as marked an effect per unit irradiation time as does ultra violet treatment and, also, that the return to the original excitability after the cessation of visible irradiation is rapid and very definite.

The above results do not indicate that a quantitative correlation can be obtained between the period of irradiation and increase in excitability. This can be attributed to the great range of the normal excitability which was found to vary from 0.33 volt to 2.33 volts. The results observed seem to indicate that preparations which are normally insensitive (a strip having a rheobase value of about 2 volts or more) show a greater percentage increase in excitability per unit time per unit intensity of irradiation than

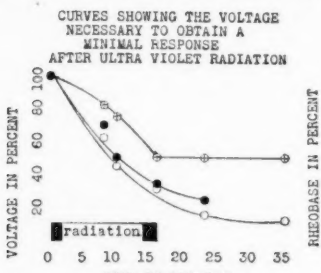

Fig. 1

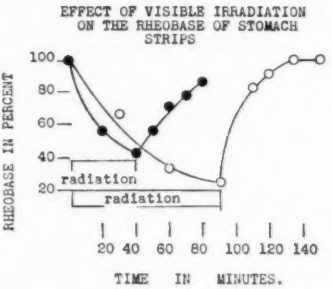

Fig. 2

Fig. 1. Curves showing the effect of ultra violet irradiation on the rheobase of stomach strips.

Fig. 2. Curves showing the effect of visible irradiation on the rheobase of stomach strips.

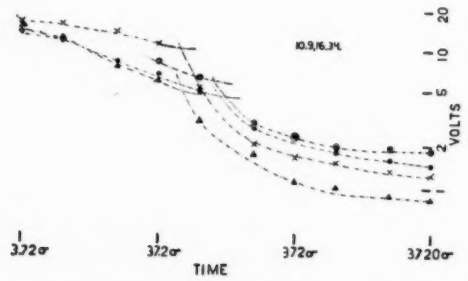

Fig. 3. Several typical strength-duration curves of stomach strips

do more sensitive preparations (having a rheobase value of 0.6 volt or less).

Strength-duration curves of smooth muscle and the effect of ultra violet and visible irradiation. Typical strength-duration curves of several untreated preparations are shown in figure 3. A break in the curves, which are plotted on a double logarithmic scale (the advantages of this procedure have been discussed by Rushton, 1931), always appeared. Grundfest

(1932), Lucas (1907) and Rushton (1930, 1931, 1932) found that strength-duration curves of striated muscle exhibited this discontinuity. They proved that this break split the curve into two parts and that the curve which appeared during long time stimulation (called the alpha curve) was one of muscle response and the curve appearing for short time stimuli (the gamma curve) was the result of the stimulation of nerve. In all probability, the break in the strength-duration curves of our preparation is also due to muscle and nerve stimulation since a nerve-muscle complex is present in the stomach strip. We believe that the curve which is found at the longer stimulation times and is slow to rise and then attains a relatively steep slope represents the muscle curve (alpha curve) and at the shorter time and higher voltages the nerve strength-duration curve (gamma curve) appears.

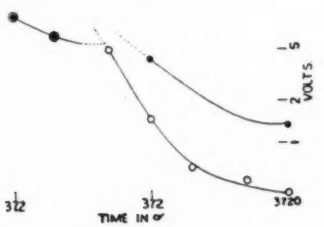

Fig. 4

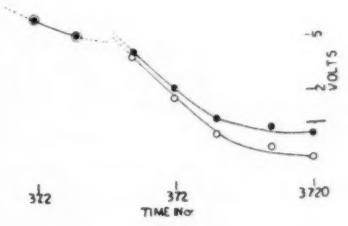

Fig. 5

Fig. 4. The effect of ultra violet irradiation on the strength-duration curve of a typical stomach strip.

—·—·—·—, control; —o—o—o, treated strip.

Fig. 5. The effect of visible irradiation on the strength-duration curve of a typical stomach strip.

—·—·—·—, control; —o—o—o, treated strip.

Numerous experiments were performed on stomach strips which were subjected to ultra violet and visible irradiation. In these experiments strips from the same stomachs were used as controls and a single experiment was always completed within two hours after dissection.

Figures 4 and 5 show typical strength-duration curves of control strips and segments subjected to ultra violet and visible radiation. Both treatments seemed to lower the alpha curve but did not alter the gamma curve as the latter curves are coincident for the treated strips and controls.

Thus, if our assumption as to the two curves (alpha and gamma) in smooth muscle is correct, we see that the irradiation which was employed does not affect nerve while the muscle curve is appreciably lowered; *i.e.*, the excitability of muscle is increased.

No definite changes in chronaxie due to irradiation were observed.

Discussion. We observed that both ultra violet and visible radiation,

without the use of fluorescent sensitisers, caused a decrease in the rheobase (*i.e.*, increased excitability) of the smooth muscle preparation employed. It was found that ultra violet produced the greater change. The above observations on the effect of visible radiation are not in accord with the findings of Adler (1920) and Azuma and Hill (1926). These investigators reported no effect of visible radiation without fluorescent sensitisers. Also, our observations conflict with those of Harris (1925) who reported a "physiological interference" between ultra violet and visible radiation. Harris found that visible radiation decreased the excitability of smooth muscle.

There is little doubt but that the various treatments employed induce certain profound changes—the excitability of smooth muscle is increased. Various investigators have reported a primary stimulation effect caused by brief radiation and a secondary deleterious effect caused by prolonged radiation of the same intensity (Packard, 1916, 1931; Warren, 1928). We are here chiefly concerned with the primary stimulation.

Ultra violet is generally regarded as influencing, by means of ionisation, the photo-active elements in the blood and skin of the higher animals, and the protoplasm of lower animals (Clark, 1922). This effect on photo-active elements is accompanied by a permeability change (Blackman and Paine, 1918; Lepeschkin, 1930 and 1933; and Tröndle, 1910) which allows a freer interchange of the various ions present. Guttman (1935), working on ultra violet radiation and its effect on the clam heart and frog heart (unpublished) subjected to excess of potassium, observed that ultra violet is able to overcome the effect of potassium excess. Thus it appears quite possible that a shift in the equilibrium between calcium and potassium, enhanced by an increased permeability, may account in part for the phenomena reported in this paper. The action of a humoral mechanism must be kept in mind. Some of our recent experiments have strongly suggested such a possibility.

CONCLUSIONS

1. The method employed (stimulation by means of the thyratron chronaximeter) in obtaining the rheobase of stomach strips appears to be quite accurate for testing the excitability of smooth muscle.

2. All strips from the frog's stomach are sensitive to radiation in the visible range as well as in the ultra violet region employed.

3. Ultra violet radiation, if applied for the same time as visible radiation and other factors being equal (such as temperature, resistance of the tissue, normal excitability, intensity of irradiation, etc.) produced a greater increase in excitability than did irradiation from the visible end of the spectrum.

4. After visible irradiation the strips soon returned to their normal

excitability. After strong ultra violet irradiation no such return to normal excitability was observed.

5. The frog's stomach shows a broken strength-duration curve, indicating a low voltage, long time branch (alpha curve) for direct muscle stimulation and a high voltage, short time branch (gamma curve) for nerve excitation.

6. Both ultra violet and visible irradiation depressed the liminal voltage on the muscle branch of the curve but showed no marked effect on the nerve branch of the strength-duration curve.

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EPINEPHRINE AND URINE FORMATION IN THE FROG

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Both oliguria and polyuria have been reported to result from the administration of epinephrine, but the data are limited to mammals. In the frog, observations have been made to find the effects of epinephrine upon the blood flow through the glomeruli, the intention being to find by what mechanism diuresis is induced by epinephrine. On reviewing these observations it was discovered that in none of them was the rate of water excretion measured in the frog with normal circulation of blood. Hence it was still doubtful whether the circulatory changes observed were at all related to the rate of urine production.

In some preliminary experiments in which the frog's rate of water excretion was being measured, it was noticed that administration of epinephrine caused sometimes a decrease and sometimes an increase in rate of water excretion. Further experiments were therefore carried out to find what the conditions were under which polyuria and oliguria, respectively, could be expected. At the same time it became important to correlate the rates of urine production with observable changes in the circulation. The observations to be made were therefore: rate of urine production over short periods of time, glomerular blood flow as seen under the microscope, and rate of heart beat. Fortunately the relations of aortic blood pressure of frogs to the administration of epinephrine have recently been worked out by Cullis and Scarborough (1932), so that other measurements upon the circulation were less necessary.

Frogs were prepared for observation by the procedure used in previous experiments (Adolph, 1935a). The brain back to the medulla was crushed, one kidney was exposed for microscopic examination, and the ureter leading from it was cannulated. The position of the urinary meniscus in the cannula was read frequently, usually at one-minute intervals. The frog and cannula were enclosed in a gas-tight chamber, and in most experiments were kept in an atmosphere of pure oxygen in order that the production of urine might be maximal (Adolph, 1935b).

In the course of the experiments it was found that very prompt responses could be obtained when the epinephrine was administered subcutaneously

as well as when administered intravenously. In addition, the introduction of extra *fluid* into the body, with or without the epinephrine, appeared to be of consequence. Each type of experiment that was performed was checked by using two different brands of epinephrine solution and controlled by administration of equal volumes of saline. All the frogs used belonged to the species *Rana pipiens.*

Subcutaneous administration. Solutions of epinephrine were administered beneath the skin in some experiments by direct injection from a syringe through a needle. In other experiments the recommendation of

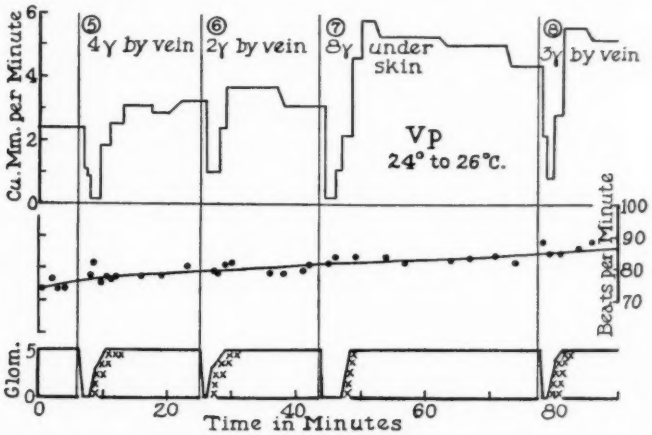

Fig. 1. Rates of urine formation, of heart beat, and of glomerular blood flow are plotted against time, in pithed frog *Vp* kept in an atmosphere of 100 per cent oxygen. The blood flow in glomeruli is represented as follows: the number of glomeruli that had some blood passing through them, out of the five glomeruli that were under continuous observation, is plotted as ordinate. Whenever the blood flow in one of them was unusually slow a cross was placed under the ordinate. The volumes of 0.1 M NaCl solution that dissolved the epinephrine and bore it into the ventral abdominal vein are indicated in table 1.

Cullis and Scarborough (1932) was carried out and a piece of fine rubber tubing was inserted beneath the skin (of the jaw region) previous to the experiment.

When injected subcutaneously in doses of 5 to 25 micrograms (γ) oliguria immediately prevailed (fig. 1, test 7). The rate of urine formation diminished within 1 minute after the injection. This surprising rapidity of response evidently depended upon the actual absorption of some of the epinephrine into the circulation, since, if 0.1 M sodium chloride solution was substituted no diminution in the rate of urine formation occurred (table 1). The promptness with which oliguria resulted after subcutaneous

TABLE 1

Tests in which epinephrine was injected into pithed frogs

TEST NUMBER	BODY WEIGHT	DOSE INJECTED	0.1 M NaCl INJECTED	ATMOSPHERE	TEMPERATURE	INITIAL CHANGES OF RATE			REMARKS
						Urine	Heart	Glomerular flow	
Subcutaneous injections									
	gm.	*γ*	*cc.*	*per cent*	*°C.*	*per cent*	*per cent*	*per cent*	
Tx 17	26	0	1.30	100 O_2	20	+5000	0	+15	After hemorrhage
18		0	1.00	100 O_2	20	+5000	+7	+10	After hemorrhage
20		0	0.50	100 O_2	20	+2500	+11	+20	Failing circulation
Uv 5	42	0	1.20	100 O_2	21	+100	+3	0	After chloroform
7		60	0.25	100 O_2	21	∝	+41	−70	After chloroform
Vi 4	37	21	0.21	100 O_2	20	−100	0	−100	After chloretone
8		21	0.21	100 O_2	20	−71	0	−35	After chloretone
Vk 2	39	0	0.85	100 O_2	22	∝		+15	Getting started
3		0	0.85	100 O_2	22	+105	+3	0	
4		25	0.40	100 O_2	21	−65	+7	−100	
5		15	1.00	100 O_2	19	−95	+5	−40	
Vl 5	39	10	0.20	100 O_2	19	−80	+6	−100	
Vp 7	39	8	0.40	100 O_2	25	−94	+2	−100	See figure 1
Vq 2	40	4.4	0.22	100 O_2	22	+27	0	−50	
4		2	0.10	100 O_2	25	+380	+5	0	
9		4	0.20	100 O_2	25	+380	+10	−25	After chloretone
Vs 2	26	0	0.87	100 O_2	24	∝	+10	0	Getting started
7		5	0.40	100 O_2	23	+1500	+30	−100	See figure 2
Intravenous injections									
Ot 3	30	2	0.20	21 O_2	22	+500	+28	+200	Getting started
5		2	0.20	21 O_2	21	+450		+300	Depressed by low O_2
6b		1.6	0.80	42 CO_2	21	+2500		+150	Depressed by CO_2
7d		2.5	0.50	0 O_2	21				Glom. already stopped
8b		2.5	0.25	11 O_2	21	−95		−40	Depressed by low O_2
9		2	1.00	21 O_2	21	+130	+6	+50	
Vp 3	39	1	0.15	100 O_2	22	−35	+3	−50	
4		2	0.10	100 O_2	24	−10	0	0	
5		4	0.15	100 O_2	24	−97	+4	−100	See figure 1
6		2	0.02	100 O_2	25	−70	0	−100	See figure 1
8		3	0.03	100 O_2	26	−82	+3	−100	See figure 1
Yg 2	42	0	0.20	100 O_2	22	+70	0	0	
3		8	0.21	100 O_2	22	−99	+9		
4		13	0.30	100 O_2	21	−98	+6	−90	
5		0	0.20	100 O_2	21	+35	+3	0	

administration of epinephrine makes it evident that absorption was almost instantaneous, time for hardly more than half a circuit of the blood intervening in some instances. There is no evidence that in such a brief inter-

val subcutaneously administered epinephrine reaches the blood stream through the lymph hearts; rather it appears to penetrate through blood vessel walls.

When the epinephrine was applied to the *outside* of the skin no effect upon urine production resulted (fig. 2, test 3). When applied to the viscera, urine production was inhibited (fig. 2, test 4); when removed slight polyuria occurred (fig. 2, test 5). Application of epinephrine solutions to the surface of the kidney inhibited or stopped the flow of blood in local glomeruli; this response also occurred with surprising promptness (0.5 to 2 min.).

The oliguria following subcutaneous injection became a complete anuria in only one experiment (*Vi*, 4) out of six (table 1). The duration of the

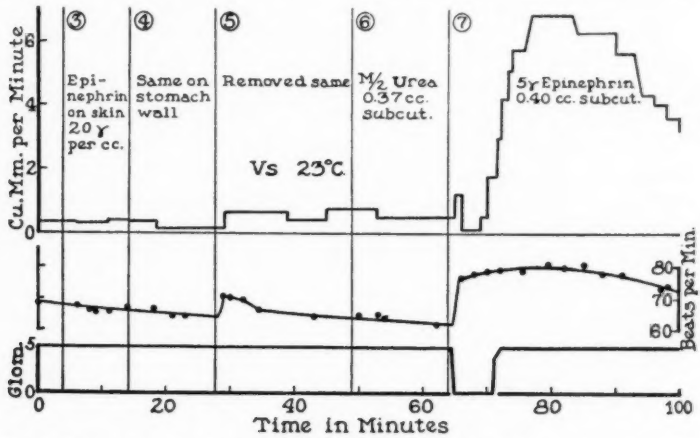

Fig. 2. Rates of urine formation, of heart beat, and of glomerular blood flow are represented as in figure 1. Pithed frog *Vs* in an atmosphere of 100 per cent oxygen. Injections were made through fine rubber tubes inserted under the skin of the lower jaw (test 6) and upper leg (test 7).

oliguria appeared to depend upon the dose administered. It could be absent altogether (with 2 to 4γ dose), be as short as 3 minutes (5 to 8γ), or last as long as 24 minutes with the largest dose (21 to 25γ). The diminution in urine rate was usually such as to prevent about four-fifths of the urine formation during the period of oliguria.

Subsequently polyuria developed. This appeared to be in response to the epinephrine rather than to the foregoing oliguria per se, and the polyuria tended to persist at gradually diminishing rates for as much as 60 minutes (fig. 1, test 7) or 100 minutes. The polyuria was most marked in animals where the rate of urine production was already very low (fig. 2, test 7). In fact, urine production could be initiated by epinephrine

administration in an animal that had been prepared but in which no urine was yet entering the ureter cannula; or in an animal that had suffered failure after a considerable period of observation and test. Experimentally the urine production could be reduced to a low rate by the previous administration of chloretone (*Vi*, 4; *Vq*, 9) or chloroform (*Uv*, 7) after which the lasting effect of epinephrine was to increase urine production enormously.

A smaller increase in absolute rate of urine formation than was characteristic of epinephrine was obtained by injecting *considerable* volumes of salt solution under the skin (0.5 to 1.3 cc. of 0.1 M NaCl to a frog weighing 26 to 42 grams). This result occurred only if the previous rate of urine production was low. At other times, the diuretic effect of epinephrine could, however, be obtained when the volume injected was only 0.1 or 0.2 cc., a similar volume of saline having little effect.

The flow of blood in some glomeruli was seen to cease simultaneously with the onset of oliguria. In other glomeruli the flow was markedly slowed. It has been found that stoppage of all renal arterial or glomerular blood flow is followed invariably by cessation of urine formation; this is the Nussbaum (1878) experiment, and appears to hold true under all circumstances. Evidently the usual persistence of some urine formation depended upon the fact that not all the glomeruli ceased to conduct blood.

The rate of heart beat increased gradually, in most cases, after the administration of epinephrine. The increase ordinarily did not appear as soon as the other changes that have been described above, and persisted for a much longer time. It was found by Cullis and Scarborough that after injection of 5γ of epinephrine at a temperature of 20°C. the increase of heart rate and aortic blood pressure lasted approximately 40 minutes and required 8 minutes to attain a maximum.

It is evident that epinephrine administered subcutaneously has two effects upon the circulation. One is to diminish temporarily the flow of blood in the arteriolar system of the kidneys. The other is to improve the circulation in general throughout the body. When some glomeruli cease to conduct blood, urine formation decreases. Polyuria prevails as soon as the flow of blood in the glomeruli becomes again active and the general circulation improves. This is particularly well illustrated in figure 2, test 7, where the improved heart rate was of no avail so long as blood had ceased to flow through all the glomeruli that were being observed.

Intravenous administration. It was supposed at the beginning of the experiments that epinephrine would result in more marked changes of urine production when given by vein than when given subcutaneously. This proved not to be the case. Three intravenous experiments are shown in figure 1; each time epinephrine was injected oliguria and stoppage of glomerular circulation both occurred for 1 to 3 minutes, followed by

polyuria which persisted. The oliguria was independent of the volume of fluid injected (table 1). The polyuria bore less relation to the volume injected than to the previously prevailing rate of urine output. Outputs that were low were more or less permanently increased many fold. The polyuria was sometimes accompanied by increase of heart rate (fig. 2) or of glomerular blood flow, but in other instances these quantities appeared to have been already maximal.

Several experiments were performed in which the rate of urine formation was first depressed by other experimental agents; these were high carbon dioxide tensions (Adolph, 1935a) and low oxygen tensions (Adolph, 1935b). When injection of epinephrine was now superimposed, marked polyuria resulted. Two such tests are shown in figure 5 of a previous paper (Adolph, 1935a). Injection of 1.6γ of epinephrine temporarily overcame the extreme oliguria due to an atmosphere of 42 per cent carbon dioxide (*Ot*, 6b) (see table 1); but 2.5γ of epinephrine did not bring about urine formation in the absence of oxygen (*Ot*, 7d), for in this condition all blood flow through glomeruli had been stopped already by local constriction of renal arterioles (Adolph, 1934). In other experiments, with oxygen tensions of 11 per cent and 21 per cent, large increases in rate of urine formation followed the injection of 2γ of epinephrine (*Ot*, 3, 5, 9). Only in an atmosphere of 100 per cent oxygen was urine formation sufficiently rapid so that it could not be enormously improved by epinephrine administration. Upon comparing the various tests upon frog *Ot* with those upon frog *Vp* it is evident that polyuria was readily obtained against a background of poor circulation, such as was produced by keeping the pithed frog in room air, while oliguria was more prominent when epinephrine was injected into a frog in good circulatory condition.

The stoppage of glomerular blood flow and the accompanying oliguria might last for as short a time as 2 minutes, but usually lasted for 4 or 5 minutes, and were followed by resumption of blood flow in glomeruli and increase of urine formation over the previous rate. The polyuria persisted for periods up to 18 minutes. Evidently epinephrine was more quickly inactivated when in the blood, than when it was being adsorbed from a lymph sac.

Changes of heart rate following the intravenous administration of epinephrine were usually absent, as shown in figure 1. In only one instance did the rate increase by more than 6 per cent. However, this moderate increase of heart rate was probably accompanied by a considerable increase of arterial blood pressure. The increased effectiveness of the systemic circulation appears to account therefore for the polyuria that persisted for periods up to an hour.

It is evident in these experiments that when epinephrine is introduced directly into the blood two effects are produced: that upon the general

circulatory system, and that upon the afferent arterioles in the kidneys. The former of these is such as to promote extra urine formation over a considerable period of time. The latter is such as to impede urine formation for a few minutes by inhibiting blood flow in the glomeruli. There appears to be no essential difference in the responses to subcutaneous and to intravenous administration of epinephrine. By the latter route smaller doses may produce oliguria, but their effect, though more intense, does not last as long as when given by the former route.

Intact frog. Many tests were required to find whether changes in rate of urine formation could be demonstrated in intact (unpithed and unoperated) frogs. In such animals the urine was caused to accumulate in the bladder by ligating the cloaca for a known period of time, while weight changes were being measured.

Twenty-four frogs were *injected subcutaneously* with 5 to 250γ of epinephrine; no significant difference in urine output or in rate of water uptake through the skin was found in comparison with ten individuals injected with an equal volume (0.20 to 0.25 cc.) of 0.1 M NaCl solution. Eighteen other frogs were injected intraperitoneally with either 50 or 250γ of epinephrine without result. When eight other frogs were similarly injected subcutaneously with 25 to 44γ, but forced to remain out of contact with water, no urine was formed in excess of the minute amounts ordinarily formed under this condition.

These negative results can easily be understood from the experiments on the pithed frogs. The brief phase of oliguria would obviously be missed in a measurement of long duration; the rate of output in the normal frog could not be measured over a period of less than 1 hour at the very shortest, and usually periods of 2 to 3 hours were used in order to obtain sufficient accuracy of the measurements.

The phase of polyuria could not be found because the normal frog already had a rate of excretion incapable of sustained improvement. An atmosphere of pure oxygen was required for a maximal rate of urine formation in the pithed frog only because such a frog was not breathing and so used unusual means of acquiring oxygen. The intact frog, therefore, behaved like a pithed frog under the very best conditions of urine formation; it was then refractory to epinephrine. It might have been supposed that a frog kept out of water would be in a condition to respond with polyuria to an injection of epinephrine, since ordinarily the urine formation is then suppressed. Such was not the case; possibly epinephrine inside the body is rapidly destroyed; clearly it is unable to overcome the factors controlling the anuria characteristic of the dry frog.

False effects of epinephrine were, however, manifested when considerable concentrations of the usual epinephrine preparations which contained chloretone were *added to the water* in which the frogs were immersed. In these experiments each frog was placed in 100 to 117 cc. of tap water.

When 10γ per cc. were given, no effect was produced (3 frogs). When 17 to 20γ per cc. were given, 4 out of 13 frogs showed almost complete retention of urine. When 30γ per cc. were given, all 8 of the frogs tested showed anuria and increase of net body weight, from which they subsequently recovered. When an equivalent amount of chloretone was used as medium for the frog (0.015 per cent solution), a similar anuria resulted (5 frogs). When 30γ of epinephrine that was free of chloretone was used per cc., no increase of net body weight appeared (2 frogs). This group of experiments was instructive because it illustrated how erroneous results may arise. The epinephrine that was *injected*, both into pithed frogs and into intact frogs, was free from chloretone.

It is evident that epinephrine cannot penetrate the body through the skin in sufficient amounts to have an effect on urine formation. When this barrier was overcome by subcutaneous injection, the effects were too temporary and too small to be detected over the period of 2 hours needed to demonstrate them. Only when urine formation was measured in short periods, as can be done solely in frogs with ureters cannulated, did either oliguria or anuria become apparent.

No effect upon the rate of water *intake* through the skin was found in either injection or immersion experiments.

Undoubtedly the amounts of epinephrine administered in many of these tests were enormously larger than any "physiological" quantities to which frogs' tissues are naturally exposed. The dose of 0.25 mgm. injected into the intact frogs weighing 30 to 40 grams is huge, and possibly equally drastic are the hundred-fold smaller doses given by vein instantaneously to the pithed frogs. It is quite doubtful whether a frog's own adrenal glands can pour out enough epinephrine to produce anuria in its own kidneys.

COMMENT. The paradoxical situation whereby epinephrine may either promote water excretion or inhibit it is now shown to depend upon the fact that epinephrine has local effects upon the arterioles of the kidneys and general effects upon the blood supply coming to the kidneys from the arteries. The local effect on the circulation within the kidneys has been noted previously, particularly by Richards and Schmidt (1924), who observed that the temporary stoppage or diminution of blood flow in glomeruli was accompanied by marked constriction in afferent arterioles; and that so long as blood flow was maintained in glomeruli, the glomeruli themselves were enlarged, as though congested with blood by the obstruction of outflow from them, perhaps by constriction of efferent arterioles. This latter observation was confirmed by Richards, Barnwell and Bradley (1927) in experiments with perfusion of the arterial system of the kidneys in bull frogs. It may be inferred that Richards believed polyuria would prevail under the conditions and at the times when his observations were made.

The effects upon pressures in the various vessels have been studied by

others. Cullis and Scarborough (1932) found that the aortic pressures of frogs increased by an average of 45 per cent when 5γ of epinephrine were given subcutaneously. Prolonged increases in arterial blood pressure due to epinephrine were reported in one frog by Bieter and Scott (1929) and in tropical toads by Lutz (1933). Hayman (1927) measured the blood pressures in the glomerular vessels of frogs by cannulating a single Bowman's capsule and imposing pressures sufficient to retard the flow of blood in these vessels. Of eight experiments in which 0.1 or 0.2 cc. of fluid containing 1 or 2γ of epinephrine were injected intravenously, five showed increases of pressure and two showed decreases. This predominance of increases was evidently due to the time at which the pressures were measured, for the pressures could not be measured while the glomerular blood flow was stopped or markedly diminished; and Hayman recognized that the pressures which he was able to measure were rarely uniform over a period of time. These facts are readily understood from the present observations of the rapidity with which the rates of urine excretion and of glomerular blood flow changed. Of all the oligurias which have been studied in the frog, that produced by epinephrine is the most transitory.

Previous observations upon rates of urine flow under the influence of epinephrine were limited to those made in *perfused* frogs and toads; Schmidt (1922), Nagasawa (1927), Kusakari and Takeda (1930) all found oliguria. The rate of arterial perfusion also decreased markedly (Richards, Barnwell and Bradley, 1927; Smith, 1927). The local action of epinephrine within the kidney vessels was apparent, therefore; but obviously by these methods that portion of the action for which the heart and arterial system are necessary could not be found. In perfusion, oliguria may be expected to persist because the supply of epinephrine is continuously renewed.

A number of previous observations have been made upon the effects of epinephrine placed on the surface of the frog's kidney. Hayman (1927), Okkels (1929), and Ebbecke and Jäger (1933) found that glomerular blood flow ceased in many units and that afferent arterioles were markedly constricted.

Thus it is evident that epinephrine in small amounts has marked effects upon the circulation of the blood in the renal arterioles and capillaries. The sites of these effects are both local, that is upon the renal afferent arterioles, and general, that is upon the heart and distant blood vessels. The two varieties of change in urine formation that are thus made possible are realized in the actual measurements of rate of water excretion; and in each specific instance the correlation is clear-cut between the circulatory changes and the changes of rate of urine formation.

SUMMARY

1. In the intact frog no significant change in rate of urine formation, lasting over a sufficient period to be measured, was induced either by injections of epinephrine or by immersion in solutions of it.

2. In the pithed frog with one ureter cannulated, the administration of epinephrine, either subcutaneously or intravenously, was ordinarily followed by extreme oliguria of brief duration. This oliguria coincided with diminution of blood circulation in the glomeruli of the kidney.

3. The oliguria was followed by polyuria of longer duration. This polyuria was correlated with improvement in the general circulation as indicated by increase of heart rate and of arterial pressure. The oliguria might fail of realization when very small doses of epinephrine were given, particularly if large volumes of salt solution were given with it, or when the rate of urine production was already small.

4. The paradox, whereby epinephrine may either promote water excretion or inhibit it, results therefore from its actions upon distinct portions of the circulatory system. Local effects in renal arterioles led to oliguria; systemic effects in the blood vessels led to polyuria.

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SALIVARY SECRETION AND THE PHYSIOLOGICAL MECHANISM OF AVITAMINOSIS-A

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The location of the pathological lesions described in vitamin A deficiency of rats bears a striking resemblance to the distribution of vasomotors thus far described in human beings. The infections of the eyes, middle ears, and sublingual glands, attributed to drying of secretions in mouth and eyes and keratinization of epithelium permitting the entrance of infections; the failure of reproduction with the characteristic changes in vaginal epithelium and sex organs; and the intestinal response which is the only symptom of such deficiency in certain animals, might all be explained by circulatory changes produced by the lack of the vitamin. The early increase in secretions, followed by decrease, and the general atrophy of organs in such deficiency also suggest changes in circulation, in water metabolism or both.

The amount of secretion obtained from a salivary fistula suggested itself as a means of measuring the progressive changes occurring during avitaminosis-A in the gland, even though it fails to distinguish between alteration in secreting cells proper and changes produced by variations in the volume of circulation through the gland.

METHOD. Fistulae were prepared for the collection of saliva in 2 dogs as described by Kleitman and Crisler (4). Subcutaneous pilocarpine was used as the stimulus to secretion, the exact amount being adjusted in each case to produce secretion without emesis. The secretion was measured for 3 successive 10 minute periods between 8 and 9 a.m. daily or every other day. The animals were fed at 4:30 p.m., long enough after the test to avoid conditioning and the water consumption, with free access to water at all times, for the preceding 24 hours was measured and corrected for evaporation.

The diet used to produce vitamin A deficiency consisted of dog biscuits made in the laboratory from a formula based on vitamin A free diet 379 of Sherman consisting of:

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper 154.

Casein, extracted for vitamin A	10 parts
Dry meat residue, free from vitamin A	10
Cornstarch	57
Crisco	10
Yeast	8
Osborne and Mendel salt mixture	4
Sodium chloride	1

A small amount of baking powder was added to give porosity, the combined materials were mixed with just enough water to form a dough, made into biscuits of about 50 grams each, and baked for an hour at 400°F. They could then be stored for a month or more. One or two drops of viosterol weekly was given to each dog. They were allowed the biscuits *ad lib*, usually 3 biscuits daily.

Bio-assay of this diet upon rats produced the typical xerophthalmia, loss of weight and final death of vitamin A deficiency, and at the same age as for rats kept in the laboratory upon Sherman vitamin-free diet 379.

To establish a norm for each dog, salivary secretion and water consumption were measured for a period of 2 weeks at the beginning of the experiment, while the dog biscuit diet was supplemented with carotene solution equivalent in vitamin A to 3 per cent codliver oil in the diet. At the end of this time carotene was discontinued for 5 months.

Results. Evidences of avitaminosis were not so marked in the dogs as in the rats fed the same diet, probably because the dogs were fully mature when started on the experiment. There was some diarrhea, however, after 2 months on the diet, which may have been due merely to pilocarpine, and some loss of appetite after 4 months. Loss of weight was only $1\frac{1}{2}$ pounds for the male dog for the duration of the experiment, but $8\frac{1}{2}$ for the female which went through a pregnancy during the experimental period. The male dog exhibited considerable inflammatory reaction in both ears, which produced great restlessness at times, and which improved on irrigation. It is impossible to say with certainty that this was due to avitaminosis and not to ear canker common in dogs.

The changes in the amount of salivary secretion were slight (table 1), the variations during progressive stages of avitaminosis being no greater than some of the daily differences. This change, however, was in the same direction as that in water consumption, namely, a slight temporary increase followed by a fall to normal or below.

The increase in water consumed in vitamin deficiency, and the change in salivary secretion, are more marked if the mean figures for the days when pilocarpine was given are considered separately from the figures for the alternate days, and in bimonthly intervals as in table 1. Figures are given for the male dog only, since pregnancy and lactation added a complicating factor in water consumption for the female. Before the pregnancy intervened, however, the water consumption for this dog also showed an increase

over the period when carotene was given. Statistically considered, the figure for water consumption immediately after carotene is withdrawn shows a difference from that when carotene was given, which is more than 4 times its probable error, thus giving odds of 142 to 1 that the same figure could be obtained again. The early increase in water consumption in vitamin A deficiency is therefore a significant one, but the difference in later stages is too small to be significant, representing merely a gradual return to normal after the early stimulation.

During the latter part of the experimental period, powdered dog biscuit was used on certain days as the stimulus to secretion instead of pilocarpine

TABLE 1

Water consumption and salivary secretion with and without pilocarpine in progressive stages of vitamin A deficiency

	WATER CONSUMPTION		SALIVARY SECRETION	
	Mean on days pilocarpine given	Mean on days without pilocarpine	Average with pilocarpine as stimulus	With food as stimulus
			cc.	*cc.*
With carotene				
December 3–19	315 ± P.E. 19.29	284	19.5	
Without carotene				
December 28–January 31	407 ± P.E. 8.75	317	20.9	
February and March	360 ± P.E. 6.82		21.6	
April and May	350 ± P.E. 10.74	284	17.2	1.6 (2.0 April) (1.3 May)
With carotene				
June	333.3 ± P.E. 23.06	289	16.0	1.4
July–August–September				1.7

to determine whether the vitamin deficiency had produced any change in the unconditioned reflex arc involved in normal salivary secretion. The amount of secretion obtained by this means was small hence small differences were not easily detected. There was apparently a decrease in such secretion in the late stages of the vitamin deficiency, with an increase when vitamin A was added; and slightly less water was consumed than on the days when pilocarpine was given. The differences are too small, however, to permit of any conclusions.

Temperature changes of the laboratory apparently bore no relation to the amount of salivary secretion, and only a very general relation to the amount of water consumed.

DISCUSSION. That inanition was not present is evident from the fact that the dogs were allowed as much food as they would eat and this remained almost constant throughout the experiment. There was only a slight temporary decline in appetite after 3 or 4 months on the diet. In the case of the female dog, in which it was most marked, this was entirely overcome by moistening the powdered dog biscuit.

Dehydration, either in the blood or tissues, as a cause for the increase in water consumption in the early part of the experiment is in line with the suggestion of Gregersen (2) that dry mouth arises in dehydration from circulatory changes following a decrease in plasma volume. Increased blood concentration in vitamin A deficiency, as measured by hemoglobin content and cell counts, has been reported by Falconer and Peachy (1) and Koessler, Maurer and Laughlin (5). Evidence for tissue dehydration in avitaminosis-A was found by the author in decreased water content of the suprarenal in rats, although the thyroid showed an increase in water content as the deficiency progressed. According to Underhill (8) such dehydration can also be produced by pilocarpine, although Kleitman (3) found no marked change in salivary secretion after prolonged use of this drug. A decrease in salivary secretion rather than the slight increase found, however, would be expected to follow dehydration, so that the change observed suggests rather a temporary central effect or parasympathetic stimulation by the vitamin deficiency.

Atrophy of glands and the consequent decrease in secretion, as suggested by Kleitman in inanition, might well explain the decline in salivary secretion, and in water consumption, as the vitamin deficiency progressed. For such atrophy in vitamin A deficiency there is the evidence of Wolbach and Howe (9) and Manville (6) who concluded that the primary effect of vitamin A deficiency is a generalized decrease in glandular activity.

In connection with the physiological mechanism of thirst, it is interesting to note that the water consumption of the dogs in this experiment was larger on the days when pilocarpine was given than on the intervening days. If thirst was due only to local dryness of the mouth, which might be expected from the epithelial changes and dehydration of vitamin A deficiency, it should have been diminished by the profuse flow of saliva produced by pilocarpine and so reduced the amount of water consumed. No such decrease in water consumption was found, however, either in this experiment or in that of Montgomery (7).

On the other hand, if dogs take water only in the hours immediately after feeding as stated by Gregersen (2), then water consumption for pilocarpine days is really represented by the figures given for alternate days and pilocarpine actually did decrease the water consumption since the water was measured before feeding. These dogs were observed often to drink immediately following the salivary test in the morning, however, which might offset the later effect of feeding on water intake.

CONCLUSIONS

The amount of salivary secretion obtained with pilocarpine from a salivary fistula in dogs shows only a slight temporary increase, probably too small to be significant, followed by a decline during progressive vitamin A deficiency; and indicates that the peripheral salivary mechanism does not suffer detectably in avitaminosis-A.

The very small changes in the salivary secretion obtained with food indicate no appreciable change in the unconditioned reflex arc.

Changes in salivary secretion are too small to serve as an index of circulatory or secretory changes occurring in avitaminosis-A.

Water consumption shows a statistically significant temporary increase followed by a decline to normal, particularly on the days when pilocarpine was given, which suggests a temporary central stimulation rather than a dehydrating effect since salivary secretion is not diminished.

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AVITAMINOSIS-A AND THE SALIVARY CONDITIONED REFLEX INDUCED BY MORPHINE

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The depressing effects of certain nutritional changes on the salivary conditioned reflex have been shown by Kleitman and Crisler (1) for inanition and by Crisler (2) for withdrawal of water. Both of these conditions are complex and include multiple mechanisms. The present paper is an attempt to test the effect of withholding only one factor, vitamin A, which is a necessary component of the mechanisms involved in inanition.

METHOD. Salivary fistulas were prepared in 4 dogs by exteriorizing the papilla of Wharton's duct (1). Control conditioned and unconditioned secretion rates for morphine were established. Vitamin A was then excluded from the otherwise adequate diet for periods up to 30 days after which vitamin A was again fed. Cameron (3), in the immediately preceding paper, has described the composition and assay of the vitamin A free diet which she supplied for these experiments. During the control periods carotene and haliver oil were used together and independently as sources of vitamin A. Water consumptions were determined daily by Cameron on other vitamin A deficient dogs kept under identical conditions with those in this experiment. The room temperature was measured daily and the dogs were weighed 3 times a week.

RESULTS. The usual individual variations between the secretion rates of different animals were evident in these experiments but all of them followed the same general trend. As shown in figure 1 for one dog, during the period when vitamin A was withheld there was a gradual depression of the conditioned secretory rate. The unconditioned secretion also decreased but not so markedly as the conditioned. The weight variation throughout the experiment was within plus or minus 1 kilo. The water consumptions did not vary significantly and secretion values did not parallel them. When vitamin A was again given, after a latent period, there was a gradual but consistent increase in the conditioned secretion. The secretion plateau after re-vitaminosis appeared to be lower than that before and in one dog the plateau remained very low until the end of the experiment, 6 weeks after vitamin A was again given.

DISCUSSION. In these experiments it must be admitted that no frank

symptoms of vitamin A deficiency were obtained. We use the term avitaminosis-A, therefore, to denote the lack of the vitamin from the diet, which was proved biologically on rats (3), rather than a distinct symptom entity.

The failure of the recovery plateau of secretion to reach the level of the plateau before the experimental period may have been caused by not enough time being allowed for full recovery. On the other hand, Kleitman and I early recognized that there is a spontaneous tendency for the conditioned secretion to decrease gradually, even though external conditions are controlled and treatment is kept constant. If the same experiment is repeated on the same animal at different times the actual secretion volumes

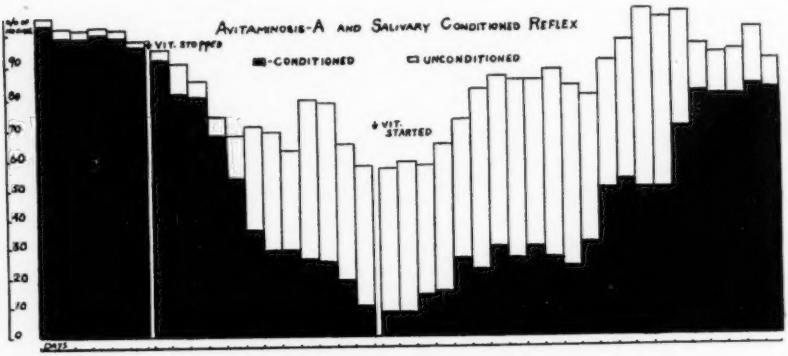

Fig. 1

may be very different although the direction of the results is the same. It is for this reason that in any given experiment it is well to express the results obtained in terms of 100 per cent for the normal.

The tendency for the unconditioned secretion to fall during the vitamin free periods of these experiments is similar to its behavior during inanition and water withdrawal. But this tendency cannot explain the almost complete abolition of the conditioned secretion because of the differences in values—a 43 per cent decrease for the unconditioned secretion against a 92 per cent decrease for the conditioned secretion. It should be pointed out again that the values which are here called unconditioned are truly mixed values. They were obtained after morphine injections but they represent the combined values of unconditioned secretion and residual conditioned secretion. If these could be accurately separated the percentage decrease in the unconditioned secretion probably would be still less than when the uncorrected values are used because of the possibility of an early relative "disappearance" of the conditioned component. The

results of Cameron (3) under similar circumstances using both a physiological unconditioned stimulus and a direct stimulus, neither of which involve conditioning, are a better indication of what happens to the normal salivary apparatus. Our results confirm her conclusions that the salivary center itself and the peripheral mechanisms are refractory to the deleterious influences of avitaminosis-A within the limits of these experiments. The avitaminosis-A must, therefore, act differentially on the conditioned center.

That the depressing influence of avitaminosis-A on the conditioned secretion is not caused by inanition is indicated by the almost constant body weights of the animals during the entire experiment. If undernutrition is a factor at all it must act selectively on nervous tissue, which is inconsistent with our belief that other evidences of undernutrition are more quickly discernible than changes in the nervous tissue.

Dehydration from a lack of drinking water is not the mechanism of depression because the water consumption is practically constant throughout. If dehydration is involved it depends upon some shift in the body economy in a direction to upset the usual relationship between water intake and tissue hydration. That such a mechanism is a possibility is shown by the reports of various authors on various tissues (4, 5, 6), but increased hydration of other tissues has also been found (6). No reports are available which show a dehydration of nervous tissue by avitaminosis-A, so that the possibility of this sort of nervous tissue dehydration being the mechanism is problematical.

Possibly these results are to be explained in terms of a non-specific effect. It is an axiom of nerve physiology that the phylogenetically younger parts of the nervous system are more sensitive to environmental changes and the first to suffer from any untoward treatment. Here we have a center phylogenetically so new that it has been developed under our observation. In all probability it is the newest integrated unit of the entire nervous system. Any effects of avitaminosis-A on the nervous system may be logically expected to influence the most sensitive part and hence, if they are untoward influences, to desynchronize if not actually destroy the integrity of the young center. Such an explanation would still leave the mechanisms of the depressing action of avitaminosis-A on the center unelucidated.

SUMMARY

Avitaminosis-A depresses the salivary conditioned reflex induced by morphine. This depression occurs before systemic evidence of avitaminosis-A.

Avitaminosis-A does not significantly influence the morphine unconditioned secretion of saliva.

Anorexia and adipsia have been ruled out as the cause of this depression.

It is suggested that the mechanism is a non-specific one depending primarily upon the sensitiveness of the new (conditioned) center.

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THE STERILITY IN RABBITS PRODUCED BY INJECTIONS OF OESTRONE AND RELATED COMPOUNDS[1]

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I. Corner (1928) demonstrated that in rabbits bilaterally ovariectomized at 14 to 18 hours after coitus the ova enter the uterus in the early blastocyst stage and degenerate. The extirpation of all corpora lutea resulted in a similar degeneration and in two of six rabbits so treated the degenerating ova were retained in the tubes at $4\frac{1}{2}$ and $7\frac{1}{2}$ days post coitum. Control excisions of non-luteal tissue had no effect upon the developing embryos, and injections of corpus luteum extracts (Allen and Corner, 1929) prevented the degeneration. Burdick and Pincus (1935) showed that the daily injection of 100 to 150 rat units of oestrone (in aqueous solution) begun on the day of mating ordinarily results in the retention of the ova in the tubes for as long as nine days, and that nearly all ova whether in tubes or uterus degenerate in the early blastocyst stage. Similarly in mice 5 R.U. of oestrone injected daily prevented the uterine entry and caused degeneration in late morula stages. The obvious deduction from these experiments is that in the early blastocyst stage in rabbits and late morula in mice the ova are dependent upon a source of nutrition derived as a result of progesterone action. Corner's ovariectomy experiments would seem to obviate the possibility that the degeneration of the blastocysts is due to a direct action of oestrone. Nonetheless it is possible that sufficient oestrone remained in the circulation and oviducts over the 54 to 60 hour period between excision and blastocyst formation to affect the blastocysts if these are peculiarly sensitive to oestrone action and the intermediate cleavage stages much less sensitive.

Accordingly we undertook as a first objective a study of the sensitivity of cleaving ova to various oestrone concentrations injected over varying periods before and after ovulation. The data of this first set of experiments are presented in table 1. The oestrone used, with certain exceptions noted in the table, was in oily solution, and injected intraperitoneally.

Rabbit ova normally enter the uterus between 67 and 75 hours after coitus (Gregory, 1930; Pincus, 1930). The oviducts of all the rabbits

[1] This investigation was aided by grants from the National Research Council Committee for Problems of Sex and the Josiah Macy, Jr. Foundation.

examined before this critical time (nos. 1, 3, 4, 5, 6, 7, 8, 10, 12, 13), with the possible exception of no. 3, showed ova of normal appearance and in the

TABLE 1

The effect of oestrone injections upon early ova

RABBIT NUMBER	DAYS INJECTED	RAT UNITS PER DAY	TOTAL RAT UNITS	HOURS AFTER COITUS KILLED	NUMBER OF CORPORA LUTEA	NUMBER OF EGGS RECOVERED	FOUND IN TUBES (T) OR UTERUS (U)	CONDITION OF EGGS
1	3 before mating	150	450	64½	8	7	T	All normal blastulae
2	3 before mating	200	600	86	10	10	T	6 degenerating, 4 almost normal blastocysts
3	3 before mating	900	2,700	24	5	5	T	Not cleaved; sperm in zona
4	3 before mating	900	2,700	24	5	5	T	All normal in 2-cell stage
5	4 before mating	150	600	67½	8	7	T	All normal blastulae
6	5 before mating	300	1,500	25	5	5	T	Normal in 2–6 cells
7	10 before mating	300	3,000	25	9	9	T	4 in 1-cell, 3 in 2-cell, 2 in 3-cell
8	15 before mating	50	750	25	9	9	T	All normal in 2–3 cells
9	20 before mating	100	2,000	86	11	10	T	All degenerating blastulae
10	2 before mating 2 after mating	200*	800	70	8	8	T	Normal blastulae
11	2 before mating 2 after mating	200*	800	96	8	6	T	Tubes tightly closed; all degenerating blastulae
12	1 after mating	150*	150	38	10	10	T	Normal in 8–13 cells
13	2 after mating	150*	300	62	8	8	T	7 normal morulas (32 to 42 cells)
14	3 after mating	150*	450	86	10	4	T	All degenerating blastocysts
						1	U	
15	3 after mating	150*	450	86	9	1	T	1 normal blastocyst in uterus; others all degenerating
						3	U	
16	3 after mating	200*	600	86	11	1	T	Degenerating blastocyst
17	3 after mating	3 cc. 0.005% NaOH	9 cc.	86½	7	7	U	All normal blastocysts
18	3 after mating	3 cc. 0.005% NaOH	9 cc.	86	9	9	U	All normal blastocysts

* Oestrone in aqueous solution.

expected state of development. The ova of rabbit 3 were uncleaved, but since the 1st cleavage generally occurs at about 22 hours after copulation

and this doe was killed at 24 hours it is possible that there was a short delay of cleavage A repetition of the treatment given this rabbit was made with rabbit 4, and perfectly normal 2-cell stages were recovered. That the oestrone injected before copulation continued to affect the oviducts for at least 86 hours after injection is demonstrated by the experiments with rabbits 2 and 9, for these does were killed at 86 hours post coitum and the ova were still retained in the tubes. This retention of the ova in the tubes according to Burdick and Pincus (1935) appears to be due to a closure of the tubo-uterine junction. We are confirmed in this opinion because it was in several cases (notably with animal 11) impossible to flush through the tubes without cutting above the junction.

In all cases where the does were sacrificed after the time of normal uterine entry (nos. 2, 9, 11, 14, 15, and 16), the degenerating ova observed were in the blastocyst stage, and this was true of ova that had entered the uterus as well as those retained in the tubes. These data demonstrate too that the critical period is rather sharply demarked, for the ova recovered at 62 to 70 hours after copulation (nos. 1, 5, 10 and 13) were all perfectly normal in appearance whereas most of the ova recovered at 86 hours or later were definitely degenerating. In the case of animal 15 one perfectly normal blastocyst was recovered from the uterus, indicating that the dosage employed was probably insufficient for complete sterilization.

Del Castilio (1932) has reported no ovulation in three rabbit does receiving 10 rat units of oestrone for 31 days preceding coitus. Although much larger total dosages were employed in these experiments, from 450 to 3000 R.U. compared with 310 in Del Castilio's experiments, no prevention of ovulation occurred in the series of pre-coital injections. Every care was taken that the does used were in good condition when injections were begun, for it is a common experience to have poorly nourished rabbit does permit mating without ovulation ensuing. For this reason we cannot be certain that Del Castilio's animals would have ovulated even without injection. The alternative possibility is that a month's injection of 10 R.U. per day is more effective than 20 days' injection of 100 R.U. per day (animal 9). In any event it is obvious from the data of these experiments that for short time injections sterilization by destruction of the embryos is easily effected whereas prevention of ovulation is difficult if not impossible (see also Leonard, Hisaw, and Fevold, 1931).

These data indicate then that the cleavage stages of the ovum are insensitive to fairly large doses of oestrone. In order to obviate the possibility that the slightly alkaline medium of the aqueous solutions was not in itself a sterilizing factor two control experiments (animals 17 and 18) were undertaken in which the does received the same total amount of oestrone-free alkaline water. These experiments show that the ova of animals so treated enter the uterus and are unimpaired.

The lethal effect of these injections upon the blastocysts may be due

either 1, to a direct action of the hormone upon the developing egg, or 2, indirectly to the failure either of a proper secretion from the uterine endometrium or of a needed supply of progesterone itself. We tested the former hypothesis first by removing three 8- to 10-cell rabbit ova from the fallopian tubes, and explanting them to a medium consisting of $\frac{1}{2}$ cc. of equal parts of blood plasma and Ringer-Locke solution of oestrone containing 6 mouse units of the hormone. These ova developed into normal blastocysts in the early growth stage after two days of culturing, and the extent of development was exactly the same as that observed in a control culture containing 5 ova. Since oestrone is so sparingly soluble in water and oestriol is much more soluble we undertook three further experiments using Ringer-Locke solutions containing varying amounts of oestriol and observed no difference between the development of experimental and control cultures. Thus ova in 4 to 8 cells grown for 24 hours in various cultures containing 4.2 to 25.2γ of oestriol developed into normal morulae as did their controls; morulae in cultures containing 4.2 to 21.0γ developed into blastocysts with clearly defined inner cell masses in 24 hours; and finally early blastocysts taken from the uterus at 80 hours post coitum were placed in cultures containing 12.6 to 25.2γ of oestriol, and these definitely expanded into blastodermic vesicles with a large cavity and normal trophoblast.

The experiments with cultured ova led us to question whether oestrone can be absorbed by the ova. It seemed possible that a vital dye coupled with oestrone might enter the ova and exert an oestrone effect. We obtained from the Schering Corporation a red azo dye prepared by coupling oestrone with the diazo compound of para-chloro-aniline. Since the dye is not soluble in water we were unable to employ it in culture, so we injected large quantities into three mated does as follows: 1, rabbit 65 received 50 mgm. of the dye (dissolved in sesame oil) at 6 hours after copulation; 5 unstained ova were recovered in the tubes at 24 hours after copulation in normal 3- to 5-cell stages; 2, rabbit 66 received 50 mgm. daily on days 3 and 4 post coitum; on the 5th day post coitum 11 corpora lutea were observed and 3 ova were recovered from the uterus; one ovum was of normal size and development and unstained; the other two ova were degenerated blastulae and unstained; 3, rabbit 67 received 50 mgm. daily on days 3 and 4, and was sacrificed on the 8th day after copulation when 11 corpora lutea were present but only two normally differentiated and unstained implanted embryos. The results with rabbits 66 and 67 indicate that the dye was partially sterilizing. Nonetheless, none of the ova were stained, indicating either an indirect action of the compound or an absorption of the dye elsewhere with sufficient uncoupling to permit a limited amount of oestrone action.

We have been unable to test the hypothesis of indirect action by experi-

ments *in vitro* due to the lack of sufficiently pure preparations of progesterone, but two further experiments are worthy of note in this connection. In one we transplanted 6 ova in the 2-cell stage into a fallopian tube of a doe in heat. Six days later two of these ova were recovered from this tube and both were degenerated early blastulae. This experiment demonstrates presumably that the endogenous oestrone is as effective a lethal agent as injected hormone. In two other does we ligatured one fallopian tube at the isthmus at 14 and 19 hours after a fertile mating respectively. Six days later 2 ova were recovered from the ligatured tube in each doe, and all four ova were degenerating in late morula or early blastula stages, whereas the ova in the unoperated side had entered the uterus and were in normal late germinal vesicle stages. The indications of these experiments are that progesterone does not act as such upon the blastocysts to induce growth and development, but that it furnishes the stimulus leading to the establishment of the necessary uterine conditions.

In order further to examine the independence of the cleavage stages we transplanted four 2-cell rabbit ova into the uterus of a mouse, ligaturing the cervical end of the uterus. Forty hours later we recovered one ovum of normal appearance in a 6-cell stage. Furthermore, we transplanted seven 2-cell mouse ova into a rabbit fallopian tube ligatured at the isthmus, and forty-four hours later recovered 4 ova in an early morula stage, and one degenerated ovum.

II. Having established to our satisfaction the ineffectiveness of oestrone as a lethal agent for cleaving ova we next proceeded to examine certain quantitative relations existing in the oestrone effect upon the developing blastodermic vesicle. The data of these experiments are presented in table 2. In all these experiments oestrone injections were made at the times stated, the does were sacrificed at either the 10th or the 12th day after copulation, and the uteri were examined for implantations. Where no implantations were found the uteri were flushed with Ringer-Locke solution and the washings examined for ova. It may be stated at once that ova were never found in these washings so that the number of implantations recorded may be taken as a true index of the embryos surviving to the implantation stage.

We attempted to determine first of all the period of maximum sensitivity to oestrone administration. The data on animals 19, 20, 21, 22, 23 and 24 indicate that if equal daily injections are begun on the first day after mating five days of injection of 200 R.U. (in aqueous solution) are necessary to ensure complete sterilization. Injections made upon the 4th day after copulation require more than 400 R.U. of hormone (animal 26), injections made upon days 4 and 5 require between 100 and 200 R.U. per day (nos. 27 and 28), whereas for days 5 and 6 (no. 30) certainly more than 200 R.U. per day are required. Courrier and Raynaud (1934) found 400 R.U.

TABLE 2

The effect of various types of oestrone injections during the preimplantation period upon the implantation ratio

ANIMAL NUMBER	DAYS AFTER MATING INJECTED	RAT UNITS INJECTED DAILY	TOTAL NUMBER OF RAT UNITS	NUMBER OF CORPORA LUTEA	NUMBER OF IMPLANTATIONS	REMARKS	
19	1	200*	200	9	9	Implantations normal	
20	1–2	200*	400	9	2	Implantations normal	
21	1–3	200*	600	7	1	Implantations normal	
22	1–3	200*	600	5	2	Implantations normal	
23	1–4	200*	800	7	1	Implantations normal	
24	1–5	200*	1,000.	10	0		
25.	4	200*	200	10	8	Implantations normal	
26	4	400	400	7	1	Implantations normal	
27	4–5	200	400	7	0		
28	4–5	100	200	9	8	3 dying; 5 normal	
29	4–6	200	600	8	0		
30	5–6	200	400	12	7	Implantations subnormal in size	
31	5–6	200	400	To term		No litter	
32	3–4	100	200	To term		Litter of four	
33	3–4	200	400	12	0		
34	3–4	150	300	10	0		100% dead
35	3–4	150**	300	11	0		
36	3–4	150‡	300	8	6	Implantations normal	
37	3–4	100	200	14	5	Implantations normal	60.3% dead
38	3–4	100	200	13	6	2 embryos subnormal	
39	3–4	75	150	8	1	Implantations normal—	87.5% dead
40	3–4	37½	75	11	1	Implantations normal	
41	3–4	37½	75	6	5	Implantations normal	72.7% dead
42	3–4	37½	75	5	0		
43	3–4	30	60	10	3	Implantations normal	
44	3–4	30	60	11	11	Average diameter of egg chambers 1.43 × 1.07	33.3% dead
45	3–4	25	50	9	7	Implantations normal	
46	3–4	25	50	10	10	Implantations normal	11.1% dead
47	3–4	25	50	8	7	Implantations normal	
48	1–5	3 cc. 0.005% NaOH	15 cc.	6	5	Implantations normal	
49	No injections			10	10	1 subnormal in size	9.8% dead
50	No injections			5	5	Implantations normal	
51	No injections			11	8	Implantations normal	
52	No injections			9	9	Implantations normal	

* Oestrone in aqueous solution (Parke-Davis Theelin).

** Crystalline oestrone in oily solution.

‡ Crystalline oestrone in aqueous solution.

injected over days 5 and 6 to be completely sterilizing whereas 320 R.U. were not. This may be due to a difference in the breed of rabbits or more probably to the method of administration, since they used divided doses.

It was noted that in certain animals injected on days 4–5 and 5–6, some of the implantations presented a subnormal appearance indicating a prolongation of the oestrone effect into the post-implantation period. In order to test this one doe (no. 31) received 200 R.U. on days 5–6 and was kept till past term. No young were born. In contrast to this, doe 32 receiving 100 R.U. on days 3 and 4 produced a litter of four normal young at term. Furthermore, in all does (except no. 38) receiving sub-sterilizing doses on days 3 and 4, both the 10-day and the 12-day implantations were quite normal in appearance. Finally both does (nos. 34 and 35) receiving 150 R.U. of oestrone-in-oil on days 3 and 4 had no implantations, and this represents the lowest completely sterilizing dosage. Implantations observed in animals injected on days 3 and 4 may therefore be taken as true survivors of the lethal effect.

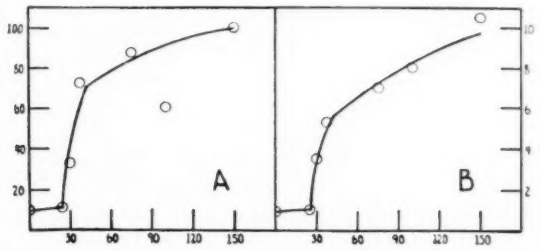

Fig. 1. Abscissa: daily dosage in R.U. on days 3 and 4 after copulation. Ordinate: A—*per cent* of embryos dead; B—number of dead embryos *per* female.

In view of the fact that 150 R.U. per day seemed to be the minimum sterilizing dosage for days 3 and 4 we were surprised to note that animal 23, receiving 200 R.U. daily from days 1 to 4 contained one normal implantation. This doe received injections of the aqueous alkaline solution of oestrone. Aqueous solutions are more rapidly absorbed and presumably more rapidly excreted than oily solutions. Moreover, Smith and Smith (1935) have recently shown that in alkaline solution the biological potency of oestrone as determined by the rat assay, is considerably impaired. We therefore dissolved crystalline oestrone in a slightly alkaline solution and determined the result when 150 R.U. daily were injected (animal 36). It will be seen that the completely sterilizing effect of an equivalent amount of oestrone-in-oil is no longer obtained. In our subsequent studies of the partial sterilization by sub-minimal amounts we therefore employed only oily solutions of oestrone.

The data on the control and injected animals are plotted in figure 1.

According to these data a definite partial sterilization is had with daily dosages of 30 R.U. and above. The 100 R.U. dosages show a lower percentage sterilization (fig. 1A) than we would expect on the basis of the other dosages, but it will be noted that the does used in these injections produced large numbers of eggs so that when the data are plotted as mean number of dead embryos per female against dosage (fig. 1B) a fairly regular increase per dosage occurs. Although these data are inadequate for a detailed consideration of the relation between dosage and lethal effect it is fairly clear that neither the percentage of embryos killed nor the absolute number is directly proportional to the dosage. On the face of it, it seems

TABLE 3

The effect of various injections of oestriol and dihydrooestrone upon the implantation ratio

ANIMAL NUMBER	DAYS AFTER MATING INJECTED	AMOUNT INJECTED DAILY	TOTAL AMOUNT	NUMBER OF CORPORA LUTEA	NUMBER OF IMPLANTATIONS	REMARKS
		gamma	*gamma*			
53	3–4	16.7*	33.3	9	8	Implantations normal
54	3–4	16.7**	33.3	9	6	Implantations normal
55	3–4	18.0**	36.0	8	0	
56	3–4	22.2*	44.4	12	0	
57	3–4	22.2**	44.4	10	0	
58	4–5	11.1*	22.2	16	16	Implantations subnormal in size
59	4–5	5.5*	11.0	10	10	Implantations normal
60	4–5	11.1*	22.2	To term		No litter
61	3–4	66.0‡	132.0	6	6	Implantations normal
62	3–4	100.0‡	200.0	7	3	Average diameters of egg chambers 1.90 × 1.43 cm.
63	3–4	150.0‡	300.0	9	2	Egg chambers = 0.8 × 1.0 and 0.9 × 1.1
64	3–4	225.0‡	450.0	8	0	

* Dihydrooestrone in aqueous solution.

** Dihydrooestrone in oily solution.

‡ Oestriol in oily solution.

that above a certain minimum, increasing the dosage results in a relative decrease of the lethal effect per embryo exposed, so that we cannot denominate a unit of dosage sufficient to kill one embryo regardless of the number of embryos. We can state that a dosage of 60 R.U. will suffice to kill at least one embryo.

III. We have undertaken briefly to compare the sterilizing potency of oestrone with that of oestriol and dihydroestrone in order 1, to determine whether the sterilization is oestrone-specific, and 2, to ascertain whether the oestrogenic and sterilizing potencies are similar. The data on these experiments are given in table 3. It is at once apparent (from the data on

animals 56 and 57) that the sterilization is not oestrone-specific. Since one rat unit is equal to $\frac{1}{3}\gamma$ of oestrone the minimum sterilizing dosage of 150 R.U. per day on days 3–4 is equal to 50γ. This means that oestrone has about one-third the potency of dihydroestrone (rabbit 55). By ordinary bioassay (spayed rat technique) oestrone has one-fifth to one-seventh the potency of dihydroestrone (Schwenk and Hildebrandt, 1933; Schoeller, Dohrn and Hohlweg, 1935). Oestriol exhibits two-ninths the potency of oestrone. According to Curtis and Doisy (1931) the maturing potency of oestriol is 6 to 7 times that of oestrone and the oestrogenic potency is one-half that of oestrone. Marrian (1931) also finds the oestrogenic potency of oestriol one-half that of oestrone; Butenandt and Hildebrandt (1931) give the ratio as 1:75. De Jongh (1934) confirms Curtis and Doisy as to the relative potencies in determining the vaginal opening of young rats and finds a similar ratio in their effects 1, upon the prostates, seminal vesicles, and connective tissue of the ampulla of the vas deferens in castrated male mice, and 2, upon the guinea pig mammary glands. We cannot, on the basis of the present data, offer any special explanation of the difference between the sterilizing potencies and other biological effects. Experiments (now in progress) on the rate of excretion of these substances should prove informative.

SUMMARY AND CONCLUSIONS

1. Injections of large amounts of oestrone into bred rabbit does both before and during the period of cleavage in no way affect the normal cleavage rate. The embryos die in early blastocyst stages and the period of maximum susceptibility to oestrone sterilization occurs during the 3rd and 4th days post coitum when the germinal vesicles are forming.

2. Large dosages of oestrone (up to 3000 R.U.) given before mating do not prevent ovulation.

3. Ova cultured *in vitro* with oestrone and oestriol added to the medium go through the cleavage stage at the normal rate. Oestriol also does not affect blastocyst differentiation *in vitro*. Ova transplanted into the fallopian tubes of a rabbit doe on heat cleave normally but die in early blastocyst stages.

4. The red vital dye made by coupling oestrone with the diazo compound of para-chloro-aniline does not enter the ova when injected in 50 to 100 mgm. dosages. Nonetheless these dosages are partially sterilizing.

5. Complete prevention of implantation is had with the daily injection of 150 R.U. of oestrone-in-oil on days 3 and 4 post coitum. Complete prevention of implantation is not had with daily dosages of 200 R.U. on days 5 and 6 post coitum. Oestrone-in-oil is more effective than oestrone in aqueous alkaline solution.

6. The injection of less than 150 R.U. per day on days 3 and 4 post

coitum results in a reduction in the number of implantations. The lower the dosage, the nearer to unity is the implantation ratio (number of implantations/number of corpora lutea), but the proportionality is not direct since lower dosages (above a minimum of 25 R.U. per day) are relatively more effective than higher dosages. The implantations observed appear normal and give rise to normal young.

7. Dihydrooestrone is approximately three times as effective as oestrone in preventing implantation. Oestriol is one-fourth to one-fifth as effective.

We are indebted to Dr. Oliver Kamm of Parke-Davis and Co. for supplies of theelin (oestrone) in aqueous and oily solution and for the crystalline theelol (oestriol) used in these experiments. To Dr. Erwin Schwenk of the Schering Corporation who furnished us with the various preparations of dihydrooestrone, with crystalline oestrone, and with the azo dye compound of oestrone, we express our gratitude. We acknowledge also the assistance of Dr. E. V. Enzmann.

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STUDIES ON THE GONAD-HYPOPHYSEAL COMPLEX IN ESTRIN-INJECTED RATS[1]

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This investigation was undertaken to correlate, if possible, the effect of estrin upon growth, gonads and accessory sex organs with the histologic picture presented by the hypophysis. That the administration of estrin results in gonadal atrophy is accepted as a fact by most workers, although Wade and Doisy (1935) were unable to obtain any interference with normal reproductive processes from long-continued injection of theelin and theelol. The mechanism of action of estrin upon the gonads is presumably by way of the hypophysis. Moore and Price, who have themselves carried out a great deal of research upon this problem, and by whom the question has recently been reviewed (1932), believe that estrin inhibits hypophyseal activity; Engle (1931) believes the effect is one of activation, while Lane (1935) takes the view that there is first an activating, later an inhibiting effect.

Our previous work (Spencer, D'Amour and Gustavson, 1932a, 1932b) has convinced us that the administration of adequate doses of estrin into immature rats always results in gonadal atrophy. In the present study, however, mature rats were employed, first, to see to what extent the mature gonad would be affected and second, because we felt that the control hypophyses of mature animals would present a more constant picture and hence render abnormalities in those of the experimental group more easily detected.

EXPERIMENTAL. The animals used are divided into two series which will be designated as series A and series B. In series A, 5 rat units of estrin were given daily for three weeks and 20 rat units daily a fourth week. The animals comprising series B were given the same dosage as the first but injection of 20 rat units daily was continued for an additional four weeks. Each series includes three groups: 1, normal males; 2, normal, virgin females; 3, ovariectomized females. Each group consisted of ten rats. Equal numbers of non-injected animals of the same type and age

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were studied as controls. The animals of group 3 and their controls had been ovariectomized eight months previously. The age of all animals at the beginning of the experiment was approximately ten months.

The estrin used was obtained from human pregnancy urine prepared by us according to the method previously described (D'Amour and Gustavson, 1930) and assayed according to the technic of Coward and Burn. Injections were made subcutaneously, the estrin being dissolved in olive oil. The animals were weighed weekly during the course of the injections and sacrificed four days after the last injection. Incidentally, the blood of these animals was tested for anti-hormone properties against estrin. The results have been published (D'Amour, Dumont and Gustavson, 1934); no anti-hormone could be demonstrated. Gonads, accessory sex structures and hypophyses were removed, weighed and fixed for histologic study. The hypophyses were placed in one of the three routine fixers, Helly's, Weigert's Mann Kopsch, as modified by Gatenby, or Kolatchew's (Nassonov), embedded in paraffin and cut at 3 and 6 micra. After Helly's fluid Martin's (1933) stain was used and proved to be the simplest to apply and gave the most consistent results. The Kolatchew fixation followed by Severinghaus' (1932) method of staining gave beautiful delineation of the Golgi, mitochondria and cytoplasmic granulations.

RESULTS. Effects upon body weight are shown in table 1 and the response of the gonads in table 2. It will be noted that only minor changes in weight result. This is in contrast with the effect upon immature animals where growth, as indicated by bone measurements, is markedly inhibited. The gonadal effects are progressive, being greater in series B, and are essentially as well marked as in immature animals. (Spencer et al., 1932b.) In the present experiment, however, the difference in weight between the experimental and control animals represents actual weight loss, not merely the failure of the gland to grow normally.

Fertility in the male is lost rather early, the last successful breeding of normal females by injected males occurred 19 days after beginning injections although motile sperm were found in the vasa of four of the ten animals in series A at the time they were sacrificed. None of the animals in series B showed any sperm whatever in the vasa.

Histologically, both testes and ovaries gave evidence of serious degenerative changes. While mitotic figures in the spermatogonia were numerous, only a few primary and no secondary spermatocytes could be found; the tubules were small and the interstitial tissue was decreased in amount. (See fig. 1.) Less apparent damage was visible in the ovaries although atretic follicles were numerous, some of the ova were apparently degenerating and there was a relative increase in interstitial tissue. These findings are also in agreement with previous observations in immature animals.

As regards the accessory sex structures, the seminal vesicles had an average weight only one-tenth that of the controls. The uterine findings were not clear-cut; there may have been a tendency for early injections to

TABLE 1

Effect of estrin administration on body weight

	4 13	4 20	4 27	5 10	5 16	5 24	5 31	6 7	6 12
Males, series A	276 9	275 4	273 5	261 0	252 7				
Males, series B	286 2	282 9	280 3	274 2	273 1	271 0	274 0	269 7	267 0
Castrate females:									
Series A	229 3	218 0	207 1	203 1	204 9				
Series B	261 7	252 0	244 1	243 6	248 0	248 9	250 7	255 2	253 5
Non-castrate:									
Females, series A	197 2	204 6	202 6	200 9	196 6				
Females, series B	227 3	235 8	234 1	232 7	234 1	236 9	227 1	229 0	230 5

TABLE 2

Effect of estrin upon sex organs

	TESTES			SEMINAL VESICLES		
	Average	Minimum	Maximum	Average	Minimum	Maximum
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Controls	2 64	2 17	2 82	1 30	1 14	1 77
Injected, series A	1 73	1 11	2 00	Not determined		
Injected, series B	0 61	0 43	1 04	0 13	0 10	0 42

	OVARIES			UTERI			
				Weights			Diameter
	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mm.*
Non-castrate controls	96	91	122	468	347	854	6 0
Injected, series A	63	59	88	551	322	917	6 5
Injected, series B	27	17	47	603	357	1,240	6 8
Castrate controls							2 0
Castrate injected, series A							6 0
Castrate injected, series B							3 8

cause an increase, continued injections a decrease, in the size of the uterus. Great individual variations, however, make a conclusion impossible. Histologically, the high degree of fibrosis which we have found to occur in pregnant animals as the result of estrin injections was not apparent.

All estrin-injected animals in series B possessed actively secreting mammary glands. The extent of mammary development, as far as size is concerned, was greatest in the males, where the pectoral glands were fully as large as those of a nursing mother; somethat less in the ovariectomized females and least of all in the normal females. A white, fatty fluid (milk?) flowed freely from cut portions of the glands. Histologic examination showed a flattened duct and end-bud epithelium, prominent vacuolization and tubules distended with fluid containing many fat droplets and free, usually vacuolated cells. (See fig. 2.) Since these animals were killed four days after cessation of injections, the possible release of pro-lactin from the hypophysis during this period might conceivably have been a factor.

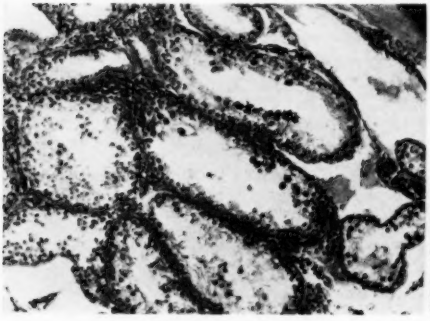

Fig. 1

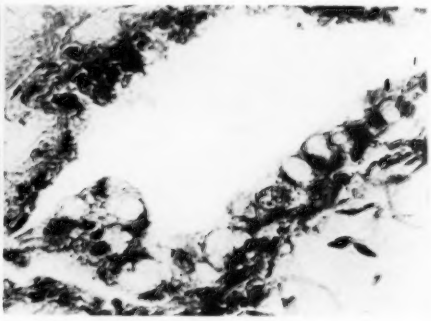

Fig. 2

Fig. 1. Testis of mature rat injected for an eight week period with estrin. A few degenerating spermatocytes are present, no spermatids nor sperm. Interstitial tissue reduced in amount. These testes (pair) weighed 0.65 gram as compared with an average weight of 2.17 grams in the control animals.

Fig. 2. Section of mammary glands of male rats following eight weeks, estrin injection, showing vacuolization of cells lining an end bud. The ducts were filled with secretion which had the appearance of milk.

Therefore the entire experiment was repeated—the dosage being as in series B-, some animals were killed immediately, others four days, and others six weeks after the injection period. Those animals sacrificed immediately showed secretory activity of the mammaries comparable in every respect with the second group and it would seem, therefore, that in the rat, estrin alone is capable of causing both proliferation and secretion. Regression of the gland was practically complete in those animals killed at the end of six weeks.

Hypophyseal findings. The weights of the hypophyses are given in table 3; it will be noted that there is a marked increase, which is progressive, being greater in series B. Histologically, the anterior lobes of normal

males and females receiving estrin are essentially alike and may be described together. There is a very marked hyperemia accompanied by an apparent increase in the blood vessels, which appear compressed and tortuous. The cells are arranged frequently in the form of narrow cords. The connective tissue has increased in amount and is now present in the form of complete septa which enclose small groups of cells. This is especially striking in sections stained with Mallory's connective tissue stain.

The enormous increase in the weight of the gland (100 to 200 per cent) is due chiefly to the hypertrophy and hyperplasia of the chromophobes. However, not all of these cells are similar to those found in the normal anterior lobe. Many are much enlarged, with an extremely large nucleus and nucleolus, and appear to be transitional between chromophobe and basophil. The nuclei show numerous mitotic figures. The average of

TABLE 3

Effect of estrin upon hypophysis

	Average	Minimum	Maximum
	mgm.	*mgm.*	*mgm.*
Control males	8 7	8 3	9 5
Injected, series A	13 4	10 2	18 0
Injected, series B	17 4	11 5	24 0
Control non-castrate females	9 0	6 0	15 5
Injected, series A	13 0	8 0	16 0
Injected, series B	28 5	17 0	42 0
Castrate controls	11 4	7 0	15 0
Injected, series A	15 5	12 0	18 5
Injected, series B	21 0	15 0	30 0

many measured nucleoli is 2.6 micra as contrasted with 1.4 micra for the normal. Accompanying these nuclear changes there are pronounced differences in the cytoplasm, Golgi apparatus, and mitochondria of the cells. The cytoplasm contains very fine basophilic granulations and resembles the cytoplasm found in the enlarged chromophobes occasionally seen in the normal gland. The Golgi apparatus is tremendously hypertrophied and in some cells occupies almost as much space as the nucleus, with the result that there remains only a small margin of cytoplasm. The Golgi apparatus is a fenestrated basket-like structure whose form and position is similar to that found in the basophiles. The mitochondria, especially conspicuous by their marked increase in size and number, are found as spherical granules scattered throughout the cytoplasm and within the meshwork of the Golgi reticulum.

The basophiles, although presenting many variable forms, appear to be

more or less completely depleted of their granules. In some the Golgi apparatus is also hypertrophied and the mitochondria are more numerous and often enlarged. Many contain a small Golgi apparatus, a few minute mitochondria and a pyknotic nucleus. There are no typically normal basophiles such as are seen in the untreated gland.

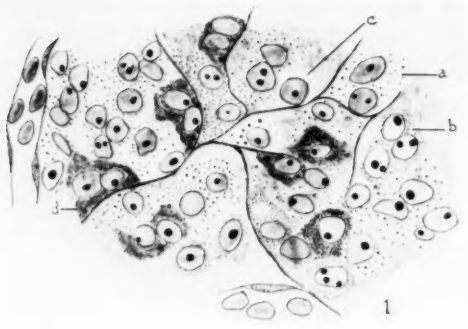

Fig. 3

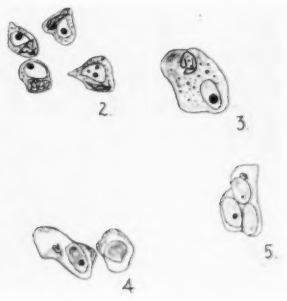

Fig. 4

Fig. 3. Explanation of figures. All figures are camera lucida drawings made at the same magnification (× 1650). The lenses used were: 2 mm. apochromatic oil immersion objective, periplan ocular number 10. The figures are reduced one-half from the original. Kolatchew (Nassonov) fixation followed by the stain of Severinghaus.

1. Field from the pituitary of a normal female that had received estrin for eight weeks. Note the compact arrangement of cells, the connective tissue septa, the hypertrophied nucleoli and the increase in size and number of the mitochondria of the basophils, *a*, and chromophobes, *b*. Basophils completely depleted of their cytoplasmic granulations are also present. Degranulated basophils, *c*. Acidophils may be distinguished by their dark cytoplasm, *d*.

Fig. 4. 2-5 are cells from the same gland as in 1. 2 shows four chromophobes with enlarged basophil-like Golgi apparatus and numerous large mitochondria. Large numbers of these cells are characteristic of the hypophyses of animals that have received estrin over a long period. 3 is of a basophil showing a general hypertrophy of cell organs. 4, degranulated basophils. 5, chromophobes with scanty cytoplasm.

The acidophiles appear to be somewhat affected. Although no cell counts were made, there seems to be some diminution in their numbers, especially in the male. The granular depletion is not as marked as in the basophiles and many of the acidophiles appear normal in all respects. (See figs. 3 and 4.)

As regards the castrate females receiving estrin, the findings in respect to blood vessels, connective tissue, nuclei, nucleoli, mitochondria and Golgi apparatus were similar to those described above. As regards the castra-

tion cells, which term we apply, following the lead of Nelson (1933), only to the fully developed cell which has the appearance of a signet ring, we found a great decrease in their number in animals of series A, and almost complete absence in series B. When present, they were much smaller than those found in the untreated hypophysis.

Discussion. Although many details are obscure, the general proposition that gonadal function is dependent upon a functioning hypophysis is universally accepted. When one encounters, as in the case of estrin-injected rats, animals whose gonads show extreme retrogression, three questions present themselves. 1. What evidence indicates that this degeneration results from lack of anterior lobe hormone? 2. Does the hypophysis itself show evidence of being affected? 3. If so, what is the mechanism of action of estrin upon the hypophysis?

1. The best evidence for believing that the gonadal atrophy is due to anterior lobe hormone starvation is obtained by comparing these findings with what takes place in the hypophysectomized animal. This comparison may be made by examining histologic sections of gonads in this series, as well as in the two studies previously reported (Spencer et al., 1932a, 1932b) and comparing them with plates published by Smith (1930) showing the effects of hypophysectomy. While space does not permit reproduction of these figures, it can be stated that, given adequate doses of estrin for a time period corresponding to the duration of hypophysectomy, gonads of both sexes show practically corresponding conditions. The extent of degeneration is greater in the case of the testis; the tubules are small, spermatocyte formation ceases and the interstitial tissue is reduced in amount. In the ovaries degenerating ova and atretic follicles are found, normal ova and corpora lutea are also present; Smith states that similar conditions prevail following hypophysectomy. Even the weight loss experienced by the gonads as a result of estrin injections agrees well with that following hypophysectomy. It seems reasonable to assume, then, that the end result of estrin injection is to deprive the gonads of a hypophyseal hormone necessary for their well-being.

The negative findings of Wade and Doisy (1935) can be explained, we believe, on the ground of inadequate dosage. The highest dosage of theelin used by these workers was 6.6 gamma daily. We have just completed a thorough assay of International Standard estrin. The results will soon be published, but it can be stated here that 6.6 gamma of this material are equivalent to only about 2 Coward-Burn rat units. If the theelin employed by Wade and Doisy was of equal potency their highest dosage would only be one-tenth of ours.

2. That the hypophysis is itself affected is indicated by the great increase in size and by the histological changes which have been described. The finding of Meyer, Leonard, Hisaw and Martin, (1932) that the hy-

pophyses of estrin-injected animals show less activity upon implantation than do normal hypophyses, points to the same conclusion. However, while the hypophysis is undoubtedly involved, the effect, in our experiment, would appear to be in the wrong direction. As was pointed out, the increased size, conspicuous mitotic proliferation, increased number of large mitochondria, hypertrophied Golgi apparatus and large nucleolus are all indicative of increased activity. We investigated the possibility that large doses of estrin might neutralize the gonad-stimulating factor, which might conceivably result in increased activity of the hypophysis without any hormone being available for the gonads. Since the results were negative the experimental data are omitted; it may be stated, however, that amounts of estrin in excess of those administered in this experiment did not neutralize the effects of gonad-stimulating factors obtained from both sheep and rat hypophyses, in the immature rat.

3. If we may conclude that the gonadal atrophy results from hormone starvation and that the injected estrin is acting through the hypophysis the question as to its mechanism of action must be raised. It is true that numerous indications of increased activity are encountered in the hypophysis. However, these criteria are not necessarily criteria of secretory activity, in fact, the basophils, commonly accepted as the cell type producing the gonad-stimulating hormone, are more or less completely depleted of their granules and the large chromophobes, which account for most of the weight increase, although containing a Golgi apparatus typical of the basophils, have not acquired the characteristic granules. The absence of granules, we believe, indicates absence of secretion. The work of Lane (1935) is suggestive in this connection. He found that the injection of estrin for a short time into normal immature females stimulated the relase of the gonadotropic hormone of the hypophysis. This was indicated by an increased number of ovarian follicles. But when estrin administration was carried out over a longer period of time the number of follicles showed a marked decrease. We believe that our cytological findings may be correlated with the physiological data of Lane, and that the first response of the hypophysis to estrin injection is an increased secretory output. However the stimulus of continued estrin injection makes demands upon it which exceed its normal productive capacity. It responds by hyperplasia. The energies of the gland seem to be devoted to proliferation rather than secretion. This is indicated by its increase in size, numerous mitotic figures, the number of incompletely differentiated cells and its decreased potency when implanted into immature animals.

We are inclined, therefore, to support Engle's (1931) view that estrin stimulates the release of gonadotropic hormone in the normal animal. This interpretation is not contradictory to the one of Moore and Price (1932), for it would seem that the apparent "depression" of the pituitary

by estrin, as judged by its decreased potency when implanted into immature animals, is merely one aspect of a reaction to intense stimulation. When the demands of the stimulus have exceeded the secretory capacity of the gland, a period of compensatory hyperplasia ensues. In this situation secretory processes become secondary to those of reproduction and growth.

SUMMARY

A study of the effect of continued estrin administration for an eight week period, into mature rats, the rats being sacrificed four days after cessation of injections, yielded the following results:

1. The gonads showed a progressive loss in weight with progressive degeneration of the germinal elements, the effect being most pronounced in males. In the animals injected for the full eight week period, this degeneration was comparable with what has been found to follow total hypophysectomy.

2. The mammary glands showed extensive proliferation and contained abundant secretion; this cannot be explained on the basis of prolactin secretion during the four days preceding examination, as a repetition of the experiment in which the animals were killed immediately gave the same results.

3. The administration of estrin leads to a marked increase in weight of the hypophysis, from 100 to 200 per cent. This increase is accounted for chiefly by the hypertrophy and hyperplasia of the chromophobes. Mitotic figures are confined chiefly to these cells; their hypertrophy is accompanied by a marked enlargement of their Golgi apparatus and nucleolus and an increase in size and number of their mitochondria. They have not acquired, however, the typical basophil granulation and many of the basophils are themselves completely depleted of their granules.

4. The injection of estrin into females castrated eight months previously results in an almost complete disappearance of the castration cell.

5. The hypothesis is advanced that estrin stimulates the release of the gonadotropic hormone in the normal animal. The "depression" of the hypophysis seen when estrin is administered is considered as one aspect of a reaction to intense stimulation. The demands of the stimulus having exceeded the secretory capacity of the gland, a period of compensatory hyperplasia follows. During this time the secretion of the gonadotropic hormone is diminished because most of the energies of the gland are now consumed by the rapid cellular proliferation.

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ABSORPTION OF WATER FROM THE SMALL INTESTINE AT VARIOUS DEGREES OF ANOXEMIA[1]

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The literature which deals with absorption from the small intestine has been reviewed by Goldschmidt (1921) and more recently by Magee (1930). The last named author, however, makes very little mention of the absorption of water.

Hamburger (1896) found that absorption of salt solutions in the intestines of dogs, dead from 1 to 24 hours, proceeded in the same manner as in living dogs. Landis (1928) reported that if experimental stasis be produced in the mesentery of the frog, the permeability of the capillary wall progressively increases. Magee and Macleod (1929) found that devitalization of the walls of segments of gut caused them to be more permeable to solutions of sugars and electrolytes than normal gut.

The experiments indicate that membranes made anoxemic, or injured, allow as much or more absorption to take place than do normal ones.

However, Brodie and his co-workers (1910) reported that during absorption from loops of intestine, there was an increase in the oxygen consumption and the carbon dioxide output.

In view of the conflicting evidence it was deemed worth while to study the problem further, and from a different angle.

METHODS. Barbitalized dogs and cats (210 mgm. per kgm.), starved for 48 hours previous to the experiment, were used. Two animals were chosen, as near the same weight and age as possible. Litter mates were used when available. One animal served as a control, and the other was subjected to anoxemia.

A mid-line abdominal incision was made. All bleeding was checked immediately, and the intestines were exposed. A ligature was placed around the small intestine at the junction of the jejunum with the duodenum, and another ligature was placed a little above the ileocecal valve. Practically the entire small intestine, then, was used as a loop, with intact

[1] The expenses of this investigation were defrayed by a grant from the Committee on Scientific Investigation of the American Medical Association.

blood and nerve supply. The loops of gut of the two animals were made of equal length, by actual measurement.

Tap water was put into the loop, by means of a large hypodermic syringe. The amount of water used depended upon the size of the animal. Care was used so as not to overdistend the gut.

After the intestinal loop of the control animal had been filled, the abdomen was closed, and the animal kept warm. The other animal was subjected to the same procedure, except that after the abdomen was closed the animal was placed in a steel respiratory chamber, previously described (Van Liere, 1927). The animals were exposed to the following oxygen percentages: 15.37, 12.28, 10.56, 8.35, and 7.03. The fluid was left in the loop for 30 minutes. At the end of that time the loops were removed and slit open, and the amount of water obtained was measured accurately. Attention was, of course, given to all details essential to the production of uniform experimental conditions.

TABLE 1

OXYGEN	INCREASE IN ABSORPTION IN ANOXEMIA	DECREASE IN CARBON DIOXIDE COMBINING POWER IN ANOXEMIA
per cent	*per cent*	*per cent*
15.37	−2.7	4.36
10.56	29.8	12.99
8.35	24.4	18.63
7.03	15.2	20.81

The carbon dioxide combining power was determined in a large number of animals. Fifteen minutes after anesthetization blood was withdrawn for a determination of the normal value; 30 minutes later, after the experimental procedures had been carried out, another sample was taken, to show the effect of the experimental conditions. In most instances duplicate determinations were made, for the sake of accuracy. All the determinations were made by one individual.

RESULTS. According to table 1 and figure 1 about as much absorption took place in the animals which were subjected to 15.35 per cent oxygen as in those which were kept at atmospheric pressure. At 12.28 per cent oxygen there was more absorption in the control animals than in those subjected to anoxemia. Those animals which were exposed, however, to more severe degrees of anoxemia, such as 10.56 per cent, 8.35 per cent and 7.03 per cent, showed a great deal more absorption than did the control animals. The greatest amount of absorption took place at 10.56 per cent oxygen.

Figure 2 shows that at 12.28 per cent oxygen the control animals in 9 groups out of 12 showed more absorption than did those exposed to anoxemia. On the other hand, at 8.35 per cent oxygen the animals which

were exposed to low oxygen tensions in 10 groups out of 11 showed more absorption than did the controls.

Table 1 shows the difference in carbon dioxide combining power as correlated with the difference in absorption. At all significant degrees of anoxemia the invariable decrease in carbon dioxide combining power is accompanied by an increased amount of absorption.

Discussion. The results reported in this paper clearly show that water is absorbed more rapidly from the small intestine when the animal is subjected to high degrees of anoxemia. In the light of our experiments, the work of Brodie (1910) is not easily explained. Our work indicates that

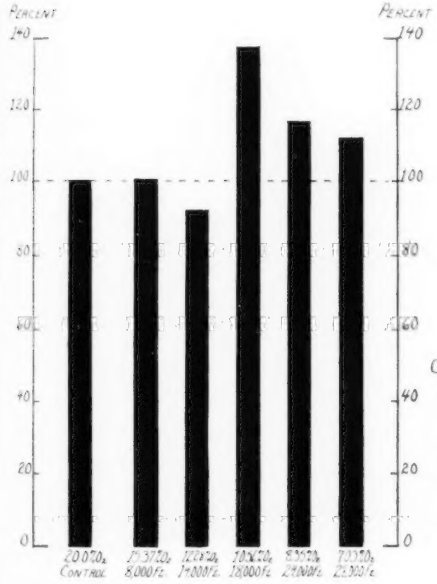

Fig. 1

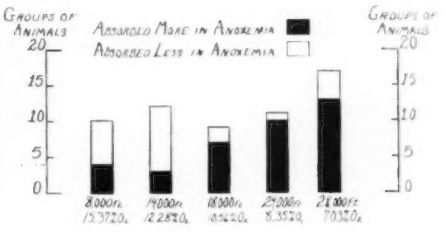

Fig. 2

absorption is more rapid when the amount of oxygen is reduced. These data are more nearly in agreement with the work of Hamburger (1896), Landis (1928) and Magee and Macleod (1929). These men did not use water in their studies, so their results cannot be too closely correlated with our own.

It is possible that the great difference in absorption between the control and the anoxemic animals at 10.56 per cent oxygen may represent an optimum. These data were obtained from 125 animals, but more data may be needed before definite conclusions could be drawn on this point.

It is difficult to explain why there is more absorption of water under

anoxemic conditions. It is known that hyperventilation induces an elevation of the blood pH and a lowering of the bicarbonate concentration. It was further pointed out by Koehler, et al. (1925) that there was always an initial alkalosis in anoxemia, and that the duration of the alkalosis was inversely proportional to the severity of the anoxemia.

As changes in the reaction of the blood in anoxemia are so constant we felt that a further study of this point might throw some light on the mechanism of absorption under our experimental conditions. The work on the carbon dioxide combining power showed that there was a definite decrease at the end of 30 minutes in those animals subjected to the higher degrees of anoxemia, while those at the less severe degrees showed a less marked decrease. In no case was any significant change in the carbon dioxide combining power encountered in any control animal.

In order to study the mechanism still further, animals were placed in the chamber at high grades of anoxemia, but were removed at the end of 14 minutes. The object was, of course, to remove them from the chamber before the acidotic stage was reached. In these animals there was no more absorption than in the controls.

It would seem from the above evidence that there is an interesting correlation between the reaction of the blood and the rate of absorption.

Fischer (1927) maintains there is more absorption from the small intestine than from the stomach because the former is much more venous. According to his theory, based on colloid chemistry, the blood richest in carbonic acid absorbs most water, since it represents an incompletely hydrated colloid.

However, this theory cannot explain all of our findings, since the greatest amount of absorption took place at 10.56 per cent oxygen, where there is a decrease of only 12.99 per cent in the carbon dioxide combining power, whereas at 7.03 per cent oxygen, where there is a decrease of 20.81 per cent in the carbon dioxide combining power, there was relatively less absorption.

Attention must also be called to the fact that injury to the cell markedly affects its permeability, as shown by Landis (1927) and others. It may well be that in severe degrees of anoxemia there is temporary injury, rendering the cell more permeable.

Further, it is questionable whether injury alone could account for all the changes encountered here. Injury would surely be in direct proportion to the degree of anoxemia, yet the animals at the most severe degrees of anoxemia showed relatively less absorption than did those at less severe degrees.

Although respirations were not recorded, it was grossly evident that there was a marked hyperpnea at higher degrees of anoxemia. However, it was obviously impossible to control any effect respiratory movements might have had. It is admitted that respiratory movements might in-

fluence the rate of absorption, but it is hard to conceive how these movements could so influence the conditions as to produce an absorption curve showing a maximum at one of the less severe degrees of anoxemia.

The work of Hamburger (1896) would indicate that circulatory factors must not be overemphasized.

The effect of anoxemia on gastro-intestinal motility as reviewed by Van Liere (1934) may be said to be one of depression of function. Hellebrandt (1934) has suggested that the mechanisms controlling motor and secretory function must be identical. King (1932) (1922) finds that prolonged and severe anoxemia is always depressant to lower spinal segments. It may well be that the motor inhibition thus produced may have some effect upon absorption.

Whether the increased permeability of the cells of the intestine, brought about by anoxemia, might be associated with the changes in the colloidal state of the blood and the depression of motility is difficult to state without more experimental evidence.

SUMMARY AND CONCLUSIONS

It was found in studying the absorption of water from the small intestine of cats and dogs at various degrees of anoxemia that animals subjected to an oxygen percentage of 15.37 showed no appreciable change from the normal. Those subjected to 12.28 per cent oxygen showed less absorption than the controls.

In those animals exposed to higher degrees of anoxemia there was considerably more absorption than in the controls. At 10.56 per cent oxygen the anoxemic animals showed 38 per cent more absorption than did the controls. Those subjected to 8.35 per cent oxygen absorbed 17 per cent more than the controls, and lastly, at 7.03 per cent oxygen, the anoxemic animals absorbed 12 per cent more than the controls.

These data suggest that there may be an optimum at an oxygen percentage of about 10.56 per cent. Work on a still larger series of animals is needed upon this particular point.

Work done upon the mechanism of the increased absorption at lowered oxygen tension indicated that the increase in absorption at higher degrees of anoxemia was correlated with a decrease in the alkalinity of the blood. Other possible mechanisms which may be involved are discussed in the body of the paper.

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THE HYPOTHALAMUS AS A SYMPATHETIC CENTER

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Evidence indicating the hypothalamus as a high sympathetic center has been slowly accumulating for some time. Leiter and Grinker (1934) cast some doubt on this belief when, after conducting experiments on ninety cats and two dogs, they concluded that the rise in blood pressure following stimulation of the hypothalamus was the result of accompanying somatic movements. Cushing (1932) and Beattie (1932) believed there were also parasympathetic centers in the hypothalamus. However, Ranson, Kabat and Magoun (1935) explored the hypothalamus thoroughly with the Horsely-Clarke stereotaxic instrument and obtained results contradictory to those of the above investigators. When stimulating the hypothalamus they obtained a rise in blood pressure, increase in rate and depth of respiration, dilatation of the pupils, contraction of the bladder, and inhibition of peristalsis. No particular region of the hypothalamus produced a single response but two or more of these responses were observed at one time, and responses were obtained from nearly the whole hypothalamus. No parasympathetic reactions were obtained from the hypothalamus except contraction of the bladder, and they believed the center controlling this reaction to be in the preoptic region. They obtained the same results on animals given sufficient amounts of curare to prevent all somatic movements.

In our use of the Horsely-Clarke stereotaxic instrument we have had the opportunity to observe the effects of electrical stimulation of the hypothalamus of twenty cats on blood pressure, heart rate, respiration and pupillary dilatation. Some regions of the subthalamus and mesencephalon were also frequently stimulated. We are reporting these results in brief mostly as a confirmation of Ranson, Kabat and Magoun's results, but also to give some additional observations of our own.

METHOD. The method used will not be explained in detail as it is essentially the same as that used by the above authors. However, most of our work was done with a unipolar electrode in the brain. We found no difference between the results of unipolar and bipolar stimulation in regard to the degree of response or the localization of response. The blood pressure in most cases was taken from the right carotid artery but in order to

eliminate the possibility of this interfering with the blood supply to the brain sufficiently to affect the results, it was taken from the abdominal aorta in several cases.

RESULTS. *Blood pressure.* Increases in blood pressure were obtained from stimulating most of the nuclei of the hypothalamus, but the most constant and greatest rises were obtained from the anterior and lateral nuclei. Good reactions were also obtained from the lateral tegmental nucleus of the mesencephalon. Descending tracts in the vicinity of this nucleus were probably being stimulated. The amount of increase from these three areas varied from 8 mm. to 70 mm., while stimulation of other areas often failed to affect the blood pressure and in no case was there a rise above 30 mm. There seems to be, therefore, a degree of localization of this function in the anterior and lateral nuclei. Variable responses from the same area were obtained in different cats but they can be partially accounted for by the varying depth of anesthesia and strength of stimulus used.

Although a drop in blood pressure occurred several times, especially after stimulating the posterior region of the hypothalamus, it occurred too seldom to be significant. Occasionally a rise in pressure on stimulation was followed by a fall below normal of short duration after stimulation was stopped. This was probably a reflex adjustment.

In some animals a slight increase in pressure was produced when the fields of Forel and the subthalamic nucleus were stimulated.

Somatic movements were observed in only a few cats and in these they were slight. Our increases in blood pressure cannot, therefore, be accounted for in this manner. Neither can they be explained as results of respiratory changes. Table 1 shows there is no correlation between changes in blood pressure and changes in respiration resulting from stimulation of the hypothalamus.

Heart rate was never significantly affected.

Respiration. Respiratory records were taken on eight cats. In seven of these there was an increase after stimulation. The reaction was varied due to depth of anesthesia, but in cat 3, in which anesthesia was light, a response was obtained from the whole hypothalamus. However, the anterior, lateral and posterior hypothalamic nuclei and the lateral tegmental nucleus of the mesencephalon gave the most pronounced results. The type of reaction was not constant. In some instances there was an increase in both rate and amplitude while in other cases there was an increase in only one phase, and the other remained normal or below normal. In two types of responses there was a localization. Rapid, deep breathing was obtained from stimulation of the anterior hypothalamic nucleus only. And rapid, shallow breathing, or panting, was obtained from the

supramammillary nucleus and the region surrounding it. Hammouda (1932) suggested the possibility of a panting center in the diencephalon.

Results from stimulating the subthalamus were very slight.

Pupillary dilatation. This was the most constant reaction obtained. It occurred from stimulations of the whole hypothalamus but the best results were obtained from the lateral area. The dilatations were usually bila-

TABLE 1

Showing variety of respiratory reactions which may accompany an increase in blood pressure

AREA STIMULATED	NUMBER OF ANIMAL	BLOOD PRESSURE	ACCOMPANYING RESPIRATORY RESPONSE	
			Rate	Amplitude
Lateral hypothalamic area	1	+20	R−	A−
		+20	R−	A+
		+12	———	
	2	+32	R+	A−
	3	+15	R++	A−
		+32	———	
	4	+14	———	
		+36	Inhibition of respiration	
		+18		A−
	5	+12	R+	A+
		+21		A+
		+24	R−	A+
	6	+40	R+	
		+16	———	
	7	+36	Rapid breathing, panting	
	8	+26	R−	A−
		+40	Inhibition of respiration	
		+12	———	
Anterior hypothalamic nucleus	3	+36	———	
		+46	R+	A++
	5	+20	———	
		+20	R−	A+
		+20	R+	A++

R+ = increased rate; R− = decreased rate; A+ = increased amplitude; A− = decreased amplitude; ——— = no effect on respiration.

teral and equal. If there was an inequality of response in the pupils the ipsilateral was always the larger. Occasionally, ipsilateral dilatation alone was produced. Some slight dilatation resulted from stimulating the subthalamic region.

We have no data to explain the mechanism of these reactions, but the suddenness of their appearance upon stimulation and cessation when stimu-

lation is stopped indicates that there is an impulse going from the hypothalamus out over the sympathetic nervous system. We agree to the plausibility of Ranson, Kabat and Magoun's hypothesis that areas in the anterior portion of the hypothalamus represent cell centers controlling these reactions, and that stimulations farther caudally may be stimulating tracts descending from these centers. The fact that responses were obtained from the lateral tegmental region of the mesencephalon, adds to the probability of this theory.

SUMMARY

1. We have confirmed Ranson, Kabat and Magoun's results showing that the hypothalamus is a sympathetic center which when stimulated produces a rise in blood pressure, increase in respiration, and dilatation of the pupils. These reactions are regulated by the hypothalamus as a whole, as there is no definite location for the control of each reaction; but better responses are obtained from the anterior and lateral hypothalamic nuclei.

2. There is some localization in regard to respiration, in that panting was produced only on stimulating the supramammary nucleus and its surrounding region, and that rapid deep respiration was produced only when the anterior nucleus was stimulated.

3. Slight rises in blood pressure and dilatation of the pupils were produced when the subthalamus was stimulated.

4. There was an increase in blood pressure and in respiration when the lateral tegmental nucleus of the mesencephalon was stimulated.

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